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9. ABSTRACT

Xerophthalmia, a result of vitamin A deficiency, now recognized for over fifty years, is still an important human affliction world-wide, particularly in children between one and six years of age. Vitamin A is not synthesized in the body and must be supplied exogenously. The occurrence precursors and metabolism of vitamin A, a micronutrient required in the diet for adequate nutrition in man and animals, are well understood and data exist on its functions, deficiency symptoms and requirements. Vitamin A can be either (a) consumed daily to meet physiological needs or (b) consumed intermittently in amounts beyond daily needs, most of the excess vitamins being absorbed, transported and stored in the liver for future tissue needs.

In an emergency or short term approach, a single massive oral or parenteral dose of vitamin A may be administered to the child twice a year for the prevention of vitamin A deficiency. For the normal or long-term approach vitamin A should be provided in the daily diet by the fortification of regularly consumed, indigenous foods of the concerned population. Past experience indicates that vitamin A may be added to a variety of liquid and dry foods without flavor problems, with adequate stability and full biological availability of the incorporated vitamin at low cost.

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Status Report







**VITAMIN A, XEROPHTHALMIA, AND BLINDNESS**  
**A Status Report in Three Volumes**

**Volume III**  
**VITAMIN A TECHNOLOGY**

by  
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**July 1973**

**Office of Nutrition**  
**Technical Assistance Bureau**  
**Agency for International Development**  
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## P R E F A C E

There is a general consensus among those working on the problem that a coordinated effort by the several concerned international donor organizations could sharply reduce the incidence of Vitamin A deficiency caused blindness.

As one of the concerned Agencies, the U. S. Agency for International Development (A.I.D.) has sponsored the preparation for its own use and for distribution to scientists and institutions engaged in Vitamin A programming a comprehensive review of the "state of the art" with respect to Vitamin A requirements, deficiencies, diagnostic criteria, ongoing programs and available technology.

Dr. Jack Bauernfeind, Dr. Wadie Kamel, Dr. Andre G. van Veen and Ms. Marjorie Scott van Veen have prepared three volumes covering Vitamin A, technology, problems and programs. Each volume is an independent effort. Titles and authors include:

A Global Survey of Mass Vitamin A Programs - W. W. Kamel

Vitamin A Problems with Special Reference to Less Developed Countries - Dr. A. G. van Veen and Ms. van Veen

Vitamin A Technology - J. C. Bauernfeind

Each volume is an expression of the author's own style, insights, and experiences focused on the area of his particular expertise. Of necessity, there is some overlap in material but such information is complementary rather than redundant.

This compilation should be considered a first step in an improved and expanded inter-agency program to combat Vitamin A deficiency worldwide. This comprehensive review can serve as a guide to A.I.D. and others interested in Vitamin A programming.

We are grateful to the authors and to Hoffman La Roche, Inc. and to the University of Illinois Medical Center for making it possible for Dr. Bauernfeind and Dr. Kamel to complete these assignments.

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Technical Assistance Bureau  
Agency for International Development  
U. S. Department of State







# VITAMIN A TECHNOLOGY

and

## XEROPHTHALMIA

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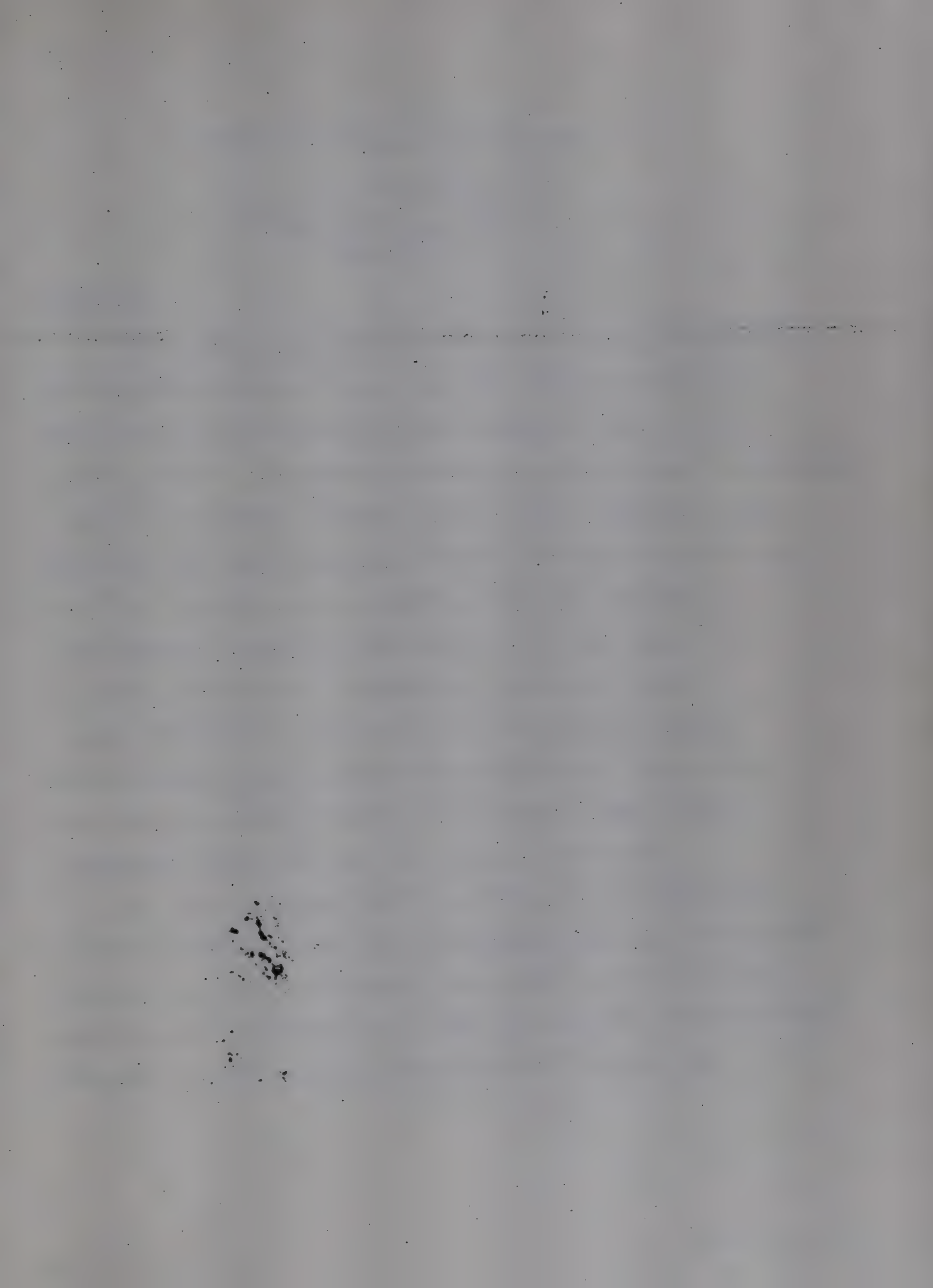


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## **Vitamin A Technology and Xerophthalmia**

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### **Introduction**

Fifty to fifty-five years have passed since the discovery that vitamin A, a fat-soluble growth factor present in liver, is a required biochemical for normal vision in man and animals and that an inadequate supply eventually results in blindness. Xerophthalmia has been a long neglected public health problem according to Patwardhan(1), Roels(2) and McLaren(9). In addition to ocular symptoms, other outward symptoms of vitamin A deficiency are growth depression and suspected greater susceptibility to disease. Absorption of dietary vitamin A is impaired in acute protein malnutrition; likewise, its mobilization and transport. Xerophthalmia is not merely a condition of recent recognition but has been recorded in early writings for which the therapy offered involved the eating of liver from an animal. For example, Eber's papyrus, an ancient Egyptian medical treatise of about 1500 B. C. , recommends ox liver or the liver of a cock as a curative agent.

The studies of Oomen et al (3 ) have indicated the wide geographical distribution of vitamin A deficiency. It is particularly and frequently associated with those areas of the world namely, Asia, Middle East, Africa, the Caribbean, and Central and South America, which most often suffer with protein-calorie deficiency. The WHO meeting on "The Prevention of Xerophthalmia





in South-East Asia", held in Hyderabad March 27-29, 1972, verified that the prevalence of vitamin A deficiency is particularly high in countries of the South-East Asian region, the Middle East and in some Latin American countries. Severe vitamin A deficiencies, particularly in children, are widespread in India, Indonesia, Thailand and the Philippines. In all Central American countries, hypovitaminosis A occurs quite frequently. The excellent nutrition survey carried out by INCAP has clearly confirmed this assumption. The vitamin A situation is critical in Brazil, mainly in the north-east region, as well as in Chile. While information on the situation prevailing in Africa is not very complete, it can be assumed that in many countries of this continent, vitamin A deficiencies are still common. In the south-west part of Africa, that is to say Ghana, Dahomey and Nigeria, the need for vitamin A represents a real problem. The condition is not rare in Turkey, Italy, or Poland or even in Western Europe. In North America, cases occur sporadically.

According to Dr. Donald S. McLaren, Professor of Clinical Nutrition at the American University of Beirut, at least 80,000 of the world's children below the age of four go blind every year and 50 per cent of these die because of a lack of vitamin A. Currently, there has been a reawakening of interest in the function of vitamin A, partly due to the growing concern over the problem of tens of thousands of children needlessly going blind from xerophthalmia every year.





During the past half century, the puzzling problem was solved of identifying, isolating, synthesizing and producing vitamin A, which exists naturally only in animal life - usually as a result of the animal's consumption of plant life containing yellow carotenoid provitamins A formed de novo in the intricate plant life physiology. The history of vitamin A is well marked with milestone achievements (Table 1).

This year, 1972, is the 25th anniversary of the production of vitamin A by chemical synthesis, one of the outstanding public health accomplishments of the period. Large volume production and current process efficiency improvements have enabled this vital biochemical to be produced in virtually unlimited quantities at a fraction of its initial cost. Yet, significant populations of parts of the world suffer because of its lack. The problems of inertia, resistance to vitamin supplementation as public health measures, unsuccessful or inadequate educational programs and particularly, distribution systems need to be overcome to wipe out blindness due to vitamin A deficiency.

#### Vitamin A Structures (Figure 1)

Vitamin A (retinol) is an unsaturated cyclic alcohol with 20 carbon atoms and five conjugated double bonds. This system of conjugated double bonds is an easy point of attack for oxygen and hence is a type of structure expected for a drying oil--but not for a vitamin. The carbon skeleton is a trimethylcyclohexenyl ring with a methylated side chain.





*Retinol* (formerly vitamin A<sub>1</sub> or axerophthol)

Empirical formula: C<sub>20</sub>H<sub>30</sub>O

Chemical name: 9,13-dimethyl-7-(1,1,5-trimethyl-6-cyclohexen-5-yl)-  
7,9,11,13-nonatetraen-15-ol

As the all-trans isomer, it possesses the highest biological activity, namely, 3,333,000 IU per gram. Vitamin A (alcohol) complexed with protein is the principal form for the vitamin in blood. Its esters are usually introduced most frequently in oral and food applications, vitamin A palmitate (1,820,000 IU/g) and vitamin A acetate (2,907,000 IU/g), manufactured by chemical synthesis. Vitamin A fatty acid esters are the liver storage form and vitamin A aldehyde (retinal) in the retina combines with protein to form the essential photosensitive pigment, rhodopsin, in the visual process of man and animals (Figure II). Vitamin A, containing 5 C=C bonds, can exist in different isomeric forms with different biological activities, the already mentioned all-trans (full activity), the 2-mono-cis (neo) or 13-cis (3/4 activity), the 6-mono-cis or 9-cis and the 2, 6-di-cis or the 9, 13-di-cis (1/4 activity). Vitamin A of the chemical synthesis origin, as manufactured commercially, consists of the all-trans isomer with a very small percentage of neo-vitamin A.





### Occurrence, Precursors and Metabolism

The natural occurrence of vitamin A is confined to mammals, birds and marine fish life; none exists in plants. In addition to vitamin A ( $A_1$ ) a second molecular variation known as  $A_2(C_{20}H_{28}O)$  exists in the tissue of fresh water fish but is biologically less active than vitamin  $A_1$ . There are other derivatives of vitamin A of lesser importance. Before the era of the chemical production of vitamin A, the principal source of vitamin A concentrates was the liver and/or body oils of marine fish (Table 2). Here vitamin A occurs both as free alcohol and predominantly in esterified form. Fish ingest carotenoids with the food plankton, lower plants, algae and crustaceans and build up liver and body oil stores of vitamin A from these food chain patterns. While plants do not contain vitamin A, apparently not requiring it, they do contain a wide array of carotenoids and at times high concentrations especially of carotenoid provitamins A, the purpose of which is presumably related to photosensitive and phototropic reactions.

Several dozen natural carotenoid vitamin A precursors are known. The more frequently recognized and the more valuable ones are probably the carctenes ( $\alpha, \beta, \gamma$ ), the apo-carotenals, the monohydroxy carotenes (cryptoxanthin), the monoketo- $\beta$ -carotenes (echinenone) and the epoxy- $\beta$ -carotenes. Palm oil is high in natural carotene content. The carotene content of natural foods is summarized in Table 3. The physiological effect of vitamin A in man and animals is brought about by a variety of compounds, varying from  $C_{20}$  to  $C_{40}$  structures.





Vitamin A of animal life can be viewed as a degradation product of the carotenoid provitamins A of plant life.

Biosynthesis of vitamin A from the carotenoid vitamin A precursors such as  $\beta$ -carotene (Figure I) takes place mainly in the intestinal mucosa during absorption by symmetric (or asymmetric) fission and/or terminal oxidation (Figure II) to produce retinal which is subsequently reduced to retinol. Orally administered vitamin A is emulsified in the small intestine by bile salts, is transformed into the alcohol form in the intestinal wall, absorbed through the wall of the small intestine, and transported by the lymph in ester form through the lymphatic duct to the liver where it is stored in the ester form. The liver has been found to contain approximately 90% of the total vitamin A in the body. When needed, the vitamin A is withdrawn from the liver and transported by the blood (in the alcohol form complexed with blood-plasma proteins) to the various tissues of the body.

Many factors influence the utilization of  $\beta$ -carotene and presumably the other carotenoid precursors as well. Since the carotenoid vitamin A precursors exist in natural foods as trans and cis isomers, in different states of oxidation depending on processing variables and stabilization techniques employed, their content and vitamin A value are difficult to know unless one (a) runs a biological assay, (b) does a chemical assay and converts to biological activity or (c) arbitrarily uses a table composition value. It is apparent that carotenoid provitamins are best utilized at quite low levels in the diet. As dietary level is increased, the efficiency of conversion to vitamin A decreases markedly. No single conversion ratio for the transformation of  $\beta$ -carotene





into vitamin A can be adopted; rather, it depends on the animal specie and condition of use. Ratios of biological effectiveness of  $\beta$ -carotene for various species including man as recommended by different authorities the USA, Canada and Britain have been summarized (Table 4) in the literature.

### Functions, Deficiency Symptoms and Requirements

The search for a general function (Table 5) of vitamin A continues since no coenzyme role has been demonstrated as in the case of the water-soluble B vitamins. Outside of the very specific role vitamin A plays in vision (Figure II), it is necessary for growth, reproduction, the maintenance of the mucus-secreting cells of the epithelia, the synthesis of glycoproteins, and the prevention of keratinization. Deficiency symptoms (Table 6) of the vitamin are quite well known, particularly xerophthalmia and night blindness. However, impaired growth and reproduction resulting from chronic or a partial deficiency, is of high concern. Over the past years the dietary vitamin A requirements (Table 7) of the various age groups for humans have become established.

### Vitamin A Synthesis and Manufacturing

All industrial syntheses of vitamin A are based on  $\beta$ -ionone (Figure III).  $\beta$ -ionone (Figure IV) is also the common intermediate in the large scale (6) synthesis of  $\beta$ -carotene and in  $\beta$ -apo-8'-carotenal. Roche synthesizes both vitamin A acetate and vitamin A palmitate as well as other forms for specific manufacturing needs. In 1947, Roche announced the first laboratory synthesis





of vitamin A. In 1950, Roche began full scale commercial production of vitamin A, the world's first commercial synthetic vitamin A production.

The Roche total synthesis of vitamin A from acetone proceeds via methylheptenone, dehydrolinalool, and pseudoionone, and twice makes use of a condensation with isopropenyl ether. Other methods of preparing methylheptenone were developed some years ago by Rhône-Poulenc (from isoprene) and BASF (from isobutylene). Lemongrass oil is no longer an economical source for citral;  $\beta$ -pinene, however, is a good starting material giving via myrcene, citral, and pseudoionone  $\beta$ -ionone (6).

The technical vitamin A syntheses by BASF, Roche, Philips, and DPi(Eastman) are shown in Figure III. It can be seen that the procedures of BASF and Roche lead directly to vitamin A acetate, which is fairly stable, crystallizes easily, and is therefore the basis of most commercially available forms. The syntheses by Philips and Eastman first give vitamin A aldehyde, which is reduced to the alcohol and only then esterified to vitamin A acetate or palmitate. In the BASF process,  $\beta$ -ionone is converted to  $\beta$ -C<sub>15</sub>-vinyl carbinol by acetylene addition and partial hydrogenation; this gives in a Wittig reaction with 2-acetoxytiglic aldehyde. vitamin A acetate. The present Roche process is, in principal, identical with the original vitamin A synthesis. It is regarded as an economical process and this view finds support in the fact that more than two-thirds of the world production is produced in this way (6).





Roche manufactures vitamin A in Nutley, New Jersey, USA, in Sisseln, Switzerland, in Dalry, Scotland and in Tana, India. Other firms also produce vitamin A in USA, France, Germany, Holland and the USSR.

The following forms of vitamin A are synthesized commercially and are available in unlimited quantities:

Vitamin A alcohol

Vitamin A acetate

Vitamin A palmitate

Vitamin A precursors

$\beta$ -carotene

$\beta$ -apo-8'-carotenal

In respect to vitamin A production, over the past several decades there has been no shortage of the synthetic vitamin and none is anticipated in the future. The production of vitamin A in the USA as recorded by the U. S. Tariff Commission is shown in Table 8. The rapid growth of world production and the impressive decline of the average price of vitamin A in logarithmic terms is illustrated in Figure V. This is a type of development which has applied, in principle, to all vitamins manufactured by chemical syntheses.





### Stability Characteristics

The stability characteristics of the vitamin A and provitamin A structures are influenced by a number of factors (Table 9).

1. Reaction with oxygen or ozone. In the absence of antioxidants vitamin A is very unstable toward oxygen. The oxidation products are ill defined.
2. Reaction with other oxidizing agents. Chemical agents having oxidizing potential are a threat to unprotected vitamin A structures. This can be demonstrated with exposure of vitamin A to oxides, dioxides and peroxides of inorganic and organic structures.
3. Instability toward acids. Vitamin A is sensitive toward acids, which can cause rearrangement of the double bonds and dehydration, eventually followed by cis-trans isomerization.
4. Heat. Thermal application to aqueous solutions and dispersions of all-trans vitamin A may result in the formation of and equilibrium of several cis isomers and trans vitamin A.
5. Moisture and trace elements. Trace metals such as copper and iron accelerate oxidative changes initiated by heat and oxygen. Moisture and pH are somewhat more critical for the acetate ester versus the palmitate ester of vitamin A in respect to maximum stability of a waterbase product.





Vitamin A is fairly stable when heated to moderate temperatures in an inert atmosphere in the absence of light, but it is unstable in the presence of oxygen or air or when exposed to ultraviolet light. Mineral acids are known to destroy vitamin A as well as its isomers. If vitamin A is treated with hydrogen chloride in ethanol, anhydrovitamin A results. In the presence of alkali, vitamin A is quite stable so that alkaline saponifications of vitamin A containing materials may be carried out without serious loss of vitamin A.

The presence of metals will also accelerate the oxidation of vitamin A. Therefore, in handling vitamin A, the three main precautions necessary are: (a) inert atmosphere (b) subdued light, (c) avoidance of trace metals and excess acid.

Surprisingly, little data have been published on the stability of vitamins in fortified foods. Much of the information obtained is in food industry's records. Available work is sketchy and difficult to extrapolate to other products because insufficient details are usually given on pH, moisture content, packaging, and other significant variables.

#### Stabilization of Vitamin A

Principal methods may be listed as follows:

- (1) Sealing under vacuum or inert gas
- (2) Storage at low temperatures
- (3) Addition of antioxidants
- (4) Sealing with a protective material such as gelatin or vegetable gums and waxes.



### 5) Complexing with other materials

The first two methods are common methods of storing any unstable compound, but they are not always convenient and practical. The use of anti-oxidants to preserve vitamin A is quite common practice. We are confined in this respect, however, to those antioxidants that are completely acceptable for food or pharmaceutical use. This list includes tocopherols, ascorbic acid and derivatives, butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and propylgallate. Antioxidants probably function by breaking up chain reactions initiated by oxygen. Once the antioxidant is used up, oxidation progresses rapidly.

The method of sealing within a matrix of gelatin is used by most vitamin A producers. In this method, the vitamin A acetate or palmitate, with or without an antioxidant, is emulsified in an aqueous gelatin-plastizer solution and then sprayed to fix the product in a beadlet form. This quickly seals the vitamin A from the air and is quite effective. The gelatin beadlet process is the basis of the most stable form of dry vitamin A on the market today. It is possible, however, that high humidities can still be eventually detrimental to a product of this type, if the physical protection of the gelatin and the chemical inhibitors are gradually disrupted and exhausted. Other methods, more of the past, include sealing within a wax or fat and wax matrix. One limitation of concern to these "sealing in" types of protection is the fact that the vitamin A must still be biologically available to the person or animal following ingestion.





Roche production facilities and experience insure that application forms of vitamin A (and vitamin A & D combinations) have good or excellent stability characteristics, by themselves (Table 10) and in the intended food application.

#### Application Forms

Over the past twenty-five years various application forms of vitamin A have been developed, a tribute to the developmental chemists and technologists to meet the wide demands of the food and pharmaceutical industries(74). In practice, vitamin A can be given as a daily supplement in food or in beverages or it can be administered by the parenteral route.

While specific vitamin A products are offered, variations can be made including the addition of vitamins D<sub>2</sub> or D<sub>3</sub> and E to customers' needs, if sizable quantities are involved. Some of the standard products are as follows:

##### A. Liquid Forms

Where high vitamin concentration is required, the following Roche liquid market forms are designed for direct incorporation into oils or the oil phase of a product.





| <u>Type and Name</u>                         | <u>Usage</u>                                                                                                                  | <u>Potency</u><br>(IU/g)     | <u>Other Ingredients</u>                                                                                                       |
|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| 1. Vitamin A Palmitate                       | Where maximum concentration is required                                                                                       | 1,650,000<br>to<br>1,800,000 | Edible antioxidant such as butylated hydroxy-anisole and butylated hydroxytoluene. No oil diluent.                             |
| 2. Vitamin A Palmitate Type PIMO             | For general use without stabilizers                                                                                           | 1,000,000                    | Edible oil only, for potency standardization                                                                                   |
| 3. Vitamin A Palmitate Type PIMO/BH          | Recommended for general use                                                                                                   | 1,000,000                    | Edible oil plus butylated hydroxyanisole and butylated hydroxytoluene as antioxidants                                          |
| 4. Vitamin A Acetate Type AIMO               | For general use without stabilizers such as oil solutions and gelatin capsules                                                | 1,000,000                    | Edible oil only, for potency standardization                                                                                   |
| 5. Vitamin A Palmitate Emulsion <sup>2</sup> | Fortification of cereal and dairy products, potato flakes, and other food products that require special processing techniques | 500,000                      | Acacia, butylated hydroxy-anisole and butylated hydroxytoluene as emulsifier and antioxidants; sodium benzoate as preservative |

#### B. Dry Products

Roche pioneered in the development of specialized dry vitamin A market forms specifically designed for different food applications.

|                                                              |                                                                                                                                                                                    |         |                                                                                              |
|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------|
| 6. Dry vitamin A Acetate Beadlets <sup>1</sup><br>Type 500   | For use in the manufacture of film or sugar coated multi-vitamin tablets or capsules                                                                                               | 500,000 | Gelatin and carbohydrate                                                                     |
| 7. Dry Vitamin A Palmitate Beadlets <sup>1</sup><br>Type 500 | For products which are not dispersed in cold water; for example, extruded foods, candies, compressed bars and similar forms where high resistance to moisture and heat is required | 500,000 | Gelatin, carbohydrate; butylated hydroxyanisole and butylated hydroxytoluene as antioxidants |



| <u>Type and Name</u>                                   | <u>Usage</u>                                                                | <u>Potency</u><br>(IU/g) | <u>Other Ingredients</u>                                                                                                                           |
|--------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| 8. Dry Vitamin A<br>Palmitate Beadlets<br>Type 250 CWS | Water dispersible;<br>for dry products<br>to be reconstituted<br>before use | 250,000                  | Gum acacia, sugar,<br>food starch-modified;<br>butylated hydroxyanisole,<br>butylated hydroxytoluene<br>and dl-alpha tocopherol as<br>antioxidants |

### C. Special Products

Application forms are in a constant state of development. As new needs arise, new forms are developed by Roche.

|                                                                   |                                                                                                                                                   |         |                                                                                                                                                                                                                        |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|---------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9. Dry Vitamin A<br>Palmitate Beadlets <sup>1</sup><br>Type 250-S | Water-dispersible,<br>free-flowing beadlets<br>to help meet the<br>stringent flavor needs<br>of milk-base products<br>and other beverages         | 250,000 | Gelatin, dextrose,<br>modified food starch,<br>coconut oil, sorbitol,<br>sodium citrate, with<br>ascorbic acid, butylated<br>hydroxyanisole, butylated<br>hydroxytoluene and<br>dl-alpha tocopherol as<br>antioxidants |
| 10. Dry Vitamin A<br>Palmitate -SD<br>Type 250-SD                 | For product<br>use wherever<br>fine particle size is<br>desirable, i. e., flour,<br>corn meal, vitamin<br>premixes, dry milk,<br>sugar, beverages | 250,000 | Acacia, lactose, coconut<br>oil; butylated hydroxy-<br>toluene, sodium benzoate<br>and sorbic acid as<br>preservatives                                                                                                 |

<sup>1</sup> Also available with added vitamin D in an A to D unit ratio of 5:1 or 10:1

<sup>2</sup> For special food-processing techniques required, these products can be made available.





## Product Specifications, Uses, Storage and Handling

Examples can be cited of further details of the above products:

### I. Vitamin A Palmitate (Retinyl Palmitate) Type PIMO

Vitamin A Palmitate, Type PIMO, Roche is all-trans retinyl palmitate diluted in corn oil. Prepared without antioxidants, the potency is 1,000,000 IU of vitamin A per gram.

#### Physical-Chemical Properties of Retinyl Palmitate

|                   |                   |
|-------------------|-------------------|
| Empirical formula | $C_{36}H_{60}O_2$ |
| Molecular weight  | 524.9             |

#### Specifications

Vitamin A Palmitate, Type PIMO meets all requirements of USP XVIII, p. 775 or revision thereof, for vitamin A, when tested according to those methods.

|                             |                                                                                                                                                  |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Appearance                  | Clear, light yellow to amber liquid with a slight, characteristic odor and a bland, oily taste                                                   |
| Solubility                  | Miscible with edible oils and fats; soluble in ether, hydrocarbons and chlorinated hydrocarbons; slightly soluble in alcohol; insoluble in water |
| Identification              | Positive for vitamin A                                                                                                                           |
| Absorbance ratio            | Minimum 0.85 at 325 mμ                                                                                                                           |
| Assay                       | Minimum 1,000,000 IU of vitamin A per gram                                                                                                       |
| Determined specific gravity | 0.90 - 0.95                                                                                                                                      |





## Uses

Vitamin A Palmitate, Type PIMO may be used wherever pure vitamin A palmitate without added antioxidants is required, e.g., in multivitamin liquids, in capsulated products, in food fortification, or in cosmetics.

This product is essentially free of (a) vitamin A alcohol; (b) various isomeric forms which have lower biological activity and (c) other vitamin A degradation products that are not biologically active.

Because the potency of this product is 1,000,000 IU of vitamin A per gram, finished product formulation and handling are simplified.

Its exceptionally high degree of purity contributes to maximum stability and biological activity.

## Storage and Handling

Store container at about 70° F for current use. Refrigerate container if stored for several months; then hold at room temperature for 24 hours before opening. After partial withdrawals, wipe can lip; close tightly. Flushing head space with purified nitrogen or CO<sub>2</sub> before closing is advisable. If contents solidify after exposure to cold, warm can in hot water bath to 140° F; rotate gently until solution is clear.



## II. Dry Vitamin A Acetate Beadlets (A Dry Preparation of Retinyl Acetate) Type 500

Dry Vitamin A Acetate Beadlets, Type 500, is a dry, free-flowing form of all-*trans* crystalline vitamin A acetate uniformly dispersed in a gelatin matrix with food-grade modified starch and sugar.

### Physical-Chemical Properties of Retinyl Acetate

|                   |                   |
|-------------------|-------------------|
| Empirical formula | $C_{22}H_{34}O_2$ |
| Molecular weight  | 328.5             |

### Specifications

|                                                                                     |                                          |
|-------------------------------------------------------------------------------------|------------------------------------------|
| Appearance                                                                          | Spherical, free-flowing beadlets         |
| Color                                                                               | Yellow to buff                           |
| Loss on drying                                                                      | Maximum 8%                               |
| Extrusion                                                                           | Maximum 5%                               |
| Identity                                                                            | Positive for vitamin A                   |
| Assay                                                                               | Minimum 500,000 IU of vitamin A per gram |
| Methods for assay and identity according to USP XVIII, p. 775, or revision thereof. |                                          |

### Characteristics of Beadlets

|       |                |
|-------|----------------|
| Odor  | Bland, typical |
| Taste | Bland, typical |

### Extrusion

The term "extrusion" describes the effect of compression pressure that forces or squeezes vitamin A from the dry beadlets during tablet





manufacture. Because this extruded vitamin A is no longer protected effectively, Roche Dry Vitamin A Beadlets have been formulated to have a minimal extrusion value.

The uniformly minimal extrusion of Dry Vitamin A Beadlets is achieved during manufacture by using specially selected and standardized gelatin, properly plasticized to provide a continuous thermoplastic seal around each uniformly distributed microdroplet of vitamin A acetate within the gelatin matrix.

#### Stability

The excellent stability of Dry Vitamin A Acetate Beadlets in bulk and in multivitamin preparations measured under a variety of stress conditions that included heat and oxidation, is attributable to (a) the high purity of the all-trans vitamin A used, (b) the composition of the matrix, and (c) the uniformly low extrusion.

#### Uses

Dry Vitamin A Acetate Beadlets are specifically recommended for use in the manufacture of film- or sugar-coated multivitamin or multivitamin/mineral tablets, chewable multivitamin tablets, and hardshell capsules.

#### Packaging and Storage

1-kilo sealed metal container  
10 - or 50-kilo fiber drum with polyethylene bag

Store in a cool, dry place.





**III. Dry Vitamin A**  
**Palmitate Beadlets**  
**Type 250 CWS**

The type CWS Beadlet is a dispersion of vitamin A palmitate in a matrix of gum acacia and carbohydrate in the form of light-yellow, free-flowing spherical granules having an irregular surface. Except for a slightly more yellow color, they are indistinguishable in physical appearance from Roche Vitamin A Acetate Beadlet.

**Physical Chemical Properties of Retinyl Palmitate**

Empirical formula       $C_{36}H_{60}O_2$

Molecular weight      524.9

**Specifications**

Appearance      Spherical, free-flowing beadlets

Color      Light Yellow

Loss on drying      Maximum 8%

Identity      Positive for vitamin A

Assay      Minimum 250,000 IU of vitamin A per gram

Methods for assay and identity according to USP XVIII, p. 775, or revision thereof.

**Dispersibility**

Even in (ice cold) water, beverages, fruit juices, milk and infant formulae, it disperses completely and quickly. The cloud of the dispersion remains uniform for relatively long periods.



### Potency

As indicated by the designation '250', the type CWS Beadlet has a labeled potency of 250,000 IU of vitamin A palmitate per gram. Extensive application of the type CWS Beadlet has demonstrated this potency to be very satisfactory for uniform distribution and stability in foods and pharmaceuticals.

### Vitamin Stability

Dry Vitamin A Palmitate, Type 250-CWS, under dry storage has about the same stability as the regular Roche Dry Vitamin A Acetate Beadlets. Samples assayed after storage at room temperature for up to one year, show vitamin A retention of about 90% or more. Vitamin A palmitate in the type CWS Beadlet exhibits better stability than does the acetate in prepared liquid dispersions. Therefore, only the palmitate is employed in the type CWS Beadlet.

### Storage

In hermetically sealed containers, gassed with nitrogen, in a cool place, protected from light and humidity.

### Uses

For dry food and pharmaceutical preparations which are reconstituted with liquids. Not for general tabletting, except effervescent tablets.





**IV. Dry Vitamin A**  
Palmitate-SD  
Type 250-SD

Vitamin A Palmitate, Type 250-SD, is the first commercially available fine particle, food grade, dry, stable vitamin A product. Its particle size range is distinctly different from conventional 40-100 mesh beadlets and it is easier to blend into minus 100 mesh food products.

**Specifications**

|            |                     |                      |
|------------|---------------------|----------------------|
| Potency    | Vitamin A Palmitate | Minimum 250,000 IU/g |
| Appearance | Fine Powder         |                      |
| Color      | Yellow              |                      |

**Ingredients**

All ingredients including antioxidants are food approved.

**Solubility**

Cold water soluble

**Stability**

Meets specifications for corn meal and wheat flour fortification issued August 30, 1968 by USDA, Agricultural Stabilization and Conservation Services, i. e. . . . . "the Vitamin A Palmitate shall have storage stability such that not more than 20 percent of its original activity shall be lost when stored for 21 days at 45°C in a sealed container at a level of 4,000 to 6,000 IU per pound in wheat flour having a moisture content in the range of 13.5 to 14.0 percent. "





Stability in flour enrichment premixes was determined by mixing 250 SD with a commercial premix (1/4 oz/100 lbs flour type) containing B<sub>1</sub>, B<sub>2</sub>, niacin and iron to produce a 70,000 IU/g product. The premix contained both reduced iron and ferric orthophosphate. The results are summarized below:

Stability in Enrichment Premix

|           |    |               |
|-----------|----|---------------|
| 6 months  | RT | 86% retention |
| 12 months | RT | 76% retention |

Biological Availability

While chemical assay procedures are available for ascertaining the vitamin A potency of a given vitamin A product, they do not indicate the biological availability of the vitamin. Three different forms of bioassay are known; the curative, the liver storage and the prophylactic. In the curative assay the animals, usually rats, are depleted of vitamin A until initial deficiency symptoms appear; then, graded levels of the standard and test substance are fed in small sub-optimal doses. In the liver storage assay, the animals are depleted of vitamin A and then relatively large doses of vitamin A are given on successive days, the animals are slaughtered and the livers assayed for vitamin A. Here also, graded levels of the standard and unknown are fed. In the prophylactic assay, the animals are fed different levels of vitamin A continuously and their growth rates measured. In all cases, a fair number of animals are used and the results evaluated statistically.



All Roche application forms during their development were subjected to various biological tests and the results used as a guide to the final adopted formulation and manufacturing procedures; hence, the vitamin A in these products are fully biologically available.

#### Analytical Methods

Physical and chemical methods of determination have the advantage of speed, precision and economy. The choice of a physico-chemical method for determining vitamin A is dictated by the nature of the sample. The most reliable method, which is applied wherever possible, is the spectrophotometric assay with appropriate correction for irrelevant absorption. This is the official method of the International British and U. S. Pharmacopoeias. It involves saponification, ether extraction, transfer to isopropanol and spectrophotometric reading at the 325 nm maximum for vitamin A alcohol and at 310 and 334 nm as a basis for the Morton-Stubbs correction. A second procedure is the Carr-Price colorimetric method which involves the reaction of vitamin A with antimony trichloride to form a blue color. At low potency levels, the spectrophotometric method can not be applied due to the extremely high non-specific absorption found in the extracts. Carr-Price procedures for the determination of vitamin A in blood and liver have been developed (Tables 11 and 12).

#### Delivery Systems for Vitamin A (7)

Fortunately, provitamin A (carotenoid precursors) vitamin A alcohol (retinol) or vitamin esters (acetate or palmitate) can either (a) be





consumed daily in amounts to meet physiological needs, or (b) vitamin A can be consumed intermittently in amounts beyond daily needs and the excess vitamin stored in the liver for future tissue needs. The carotenoid precursors, such as  $\beta$ -carotene, are less efficiently utilized than the true vitamin A compounds (5). While provitamin A, vitamin A and vitamin E are fat-soluble compounds, water-miscible liquid and dry formulations have been developed. Neither vitamin A or E are synthesized in the body and must be supplied exogenously. Manifold delivery systems have been developed to provide supplementary vitamin A for man. These can be divided into two principal categories:

I. Emergency or short term measures

- A. massive oral dose, 2-4 x a year
- B. massive parenteral dose, 2-4 x a year

II. Normal or long-term measures

- A. Fortification of a regularly consumed indigenous food by the target population
- B. Fortification of a regularly consumed beverage by the target population
- C. Introduction of a special dietary supplement to be added to the present food or beverage supply or consumed directly
- D. Encouragement to grow and consume plant foods rich in provitamin A if regularly available at low cost





### A. Oral (7)

For oral dosing of infants, children and adults with vitamin A, present vitamin technology provides a variety of vitamin product forms for the purpose. Vitamin A ester can be employed (a) in a sealed gelatin capsule, or (b) in an oil solution, or (c) in a water-dispersible liquid form. The latter two are usually added to fat and water-base foods, respectively. It should be noted, however, that (d) a water-emulsifiable dry gelatin-sugar matrix of vitamin A exists which can be made into chewable flavored tablets or added to foods as well as (e) water-emulsifiable vitamin A ester in a dry matrix which is dispersible in hot or cold water and beverages. Because of the different technologies involved, there is some variation in cost of the different product forms. All can be obtained alone or with vitamin E or D if desired.

### B. Parenteral (7)

For parenteral dosing of infants and children at doses of 100,000 IU of vitamin A, sterile aqueous dispersions of vitamin A esters can be produced for this purpose. For wide application they are prepared at a concentration of 100,000 IU of vitamin A per ml (range 50,000 to 250,000 IU) to be injected intramuscularly (IM). These have been developed based on the use of product development technology and the biological response when injected into animals such as liver storage performance. Figure VI shows the rapid response of a single intramuscular injection of 50,000 IU of vitamin A palmitate (in a water base parenteral) into a 200 g chicken with time.



In addition to oil solution, liquid emulsions and dry water-dispersible preparations of vitamin E ( $\alpha$ -tocopheryl acetate) are available for the production of sterile aqueous dispersions of  $\alpha$ -tocopherol and/or the acetate at a concentration of 50 IU per ml to be injected IM.

#### Intermittent Massive Dosing in Animals (8)

The concept of administering large or massive doses of vitamin A at definite periods was initiated with animals and is currently practiced in farm animal husbandry and management programs today. Over a decade ago sheep on pasture under drought or semi-drought conditions, receiving little or no vitamin A from their feed supply, were administered massive oral doses of vitamin A in oil solution from a drenching gun or syringe. Nursing and weaned lambs at two months or more of age were given 500,000 IU of vitamin A to last a six month or more period. As a special purpose use, massive dosing of vitamin A with beef cattle was first tried with an oral vitamin A bolus, which gradually changed to intraruminal injection and finally to IM injection. Amounts of 1,000,000 to 4,000,000 IU of vitamin A are parenterally administered as a single IM dose of water-dispersible forms in growing fattening cattle when normal diet supplementation is not convenient, to serve the animal's needs for a two to six month's period. It is also used on mature breeding animals. Vitamin A water-dispersible parenteral forms have been used with technical success and good biological efficacy in cats, dogs, rabbits, turkeys, pigs, lambs, calves, cattle and horses (8).





In any developing countries where present daily food supplies do not provide an adequate vitamin A intake to prevent blindness in children and adults, the intermittent massive dose concept is a practical interim approach to adequate vitamin A health until provision is made to supply this essential vitamin in foods widely consumed in the respective region by the nutritionally deficient population.

#### Massive Oral Dosing of Vitamin A in Children (7)

McLaren (9) in 1964 suggested intermittent massive vitamin A dosing of infants as prophylaxis against xerophthalmia within their first few months of life. Another suggestion was to treat the mother at delivery to improve vitamin A content of the colostrum and early milk. Ajans, et al (10) observed the effect of a single massive oral dose of vitamin A (600,000 IU) in water-dispersible form given to pregnant women shortly before delivery. Colostrum and early milk were collected and vitamin A values were found to remain elevated over untreated control for about 30 days following parturition. Since young children are most subject to xerophthalmia, even commendable as it is to have the nursing mother nutritionally adequate in vitamin A, the child also must begin to have vitamin A added to its diet after the first few months of age.

Intermittent massive vitamin A treatment to children has been tried by a number of investigators over the past seven years. McLaren et al (11) in 1965, orally administered 50,000 IU of water-dispersible vitamin A per kg of body weight in five divided doses over a consecutive five day period to undernourished children exhibiting some degree of ocular involvement. A





good response in serum vitamin A and a decrease in eye symptoms was obtained.

Pereira et al (12 ) reported on oral dosing with water-miscible vitamin A (100,000 IU) in kwashiorkor and normal children. While an increase in serum vitamin A values were obtained in all cases, some of which were quite rapid, the rise was not uniform throughout the group and the response was judged erratic by the authors. A single oral dose of an oil solution (100,000 IU) maintained the serum content of vitamin A of healthy preschool children higher than those of the control for a 13 week period and of vitamin A depleted children for a 15 week period (13 ). A single oral dose of 50,000 IU of vitamin A in oil solution, given to preschool children was only effective for a lesser period of time (14 ). Pereira et al (12 ) concluded that the oral administration of a low dose of vitamin A in oil solution is an unsuitable form of therapy for the initial treatment of the child with kwashiorkor and severe vitamin A deficiency because of the slow absorption of the vitamin in these malnourished children.

A single oral dose of 100,000 IU of vitamin A in oil solution was administered by Susheela (15) to children between the ages of two and six years and elevated blood serum vitamin A values were observed at four months after dosing. Reddy and Srikantia (16 ) also administered a single oral dose of vitamin A (100,000 IU) in an oil solution which resulted in a considerable increase in the concentration of the serum content of vitamin A 24 hours after the dose. The same workers (17) have extended their studies treating both normal children and those recovering from protein-calorie malnutrition, administering to both a single oral dose of 300,000 IU of



vitamin A in an oil solution. Significant blood serum values for vitamin A were maintained for a six month period and in some patients, for about a year.

Based on these previous studies, a field prophylactic trial (18) was undertaken in rural areas around the city of Hyderabad, India, on 1785 children between one and five years of age. A dose of 300,000 IU of vitamin A in an oil solution was administered orally and repeated once a year. While the program was quite successful in maintaining children symptom-free, Swaminathan et al recommended a single massive oral dose of 200,000 IU given every six months as a preferred alternate treatment in future studies. For practical consideration, Gopalan (19) also recommends two annual oral doses of 200,000 IU of vitamin A, each at intervals of six months.

In two states of India, Kerala and Mysore, 200,000 IU of vitamin A in an oil solution were administered by mouth every six months to 400,000 children between one and four years of age. The incidence of xerophthalmia was decreased by 75% by this practice. In a similar study carried out in Indonesia involving 473 preschool children, the incidence of ocular manifestations was found to be 25% of the pretreatment prevalence at the end of six months following the administration of an oral dose of 200,000 IU of vitamin A in an aqueous emulsion form (20).

Chopra and Kevany (21), expressing their views on hypovitaminosis in Central and South America, recommended 100,000 IU of water-dispersible vitamin A be administered orally 1-4 times a year for at least two years to





all children from the third month of life on, in areas where avitaminosis is a public health problem. Lactating mothers would be given an oral dose of the above preparation for the protection of the nursing child.

#### Massive Parenteral Dosing of Vitamin A in Children (7)

Instances have been reported wherein parenteral administration of oil solutions of vitamin A have been employed in humans with poor or partial efficacy. Cienfuegos (22) injected vitamin A (10,000 IU/kg body weight) in sesame oil into the muscles of the buttocks of children without significantly increased serum vitamin A values. A dose of vitamin A palmitate (600,000 IU) in oil given as a single IM injection to women at parturition failed to increase the vitamin A content of early colostrum whereas oral administration was significantly effective according to Ajans et al (10). McLaren et al (11) administered a single dose of vitamin A palmitate (50,000 IU/kg body weight) in an oil solution IM to children with some degree of ocular involvement. The response judged by blood serum values was less marked than oral administration, but both methods produced some improvement in ocular lesions.

A study was made by Pereira et al (12) of the efficacy of parenteral vitamin A, namely, vitamin A acetate in oil (40,000 to 100,000 IU given each child daily for four to seven days) and water-miscible vitamin A palmitate (a single dose of 100,000 IU) to children with kwashiorkor and early keratomalacia and to normal children. With the water-miscible vitamin A, ocular symptoms began to decrease overnight and blood serum vitamin A





values began to rise one to two hours following the dose, while with the oil solution, a delayed response of eye symptom improvement was noted and erratic blood serum increases were observed with time. Confirmation of the lower efficiency of the parenteral administration of oil solutions of vitamin A was obtained in the study of Pereira and Begum (23 ) wherein IM injection of vitamin A in oil in single doses of 100,000 to 300,000 IU failed to maintain serum values in normal preschool children. As a result of their studies, Pereira et al (12 ) adopted the single IM injection water-miscible vitamin A (100,000 IU) for children with kwashiorkor and severe vitamin A deficiency symptoms, followed by intermittent oral treatments of vitamin A in oil as the patients progress. They also caution, however, of the potential danger of hypervitaminosis A using highly effective water-miscible vitamin A.

Water-miscible vitamin A palmitate in a singular parenteral dose (100,000 IU) was administered by Srikantia and Reddy (17 ) to children between eighteen months and four years of age suffering from various grades of protein-calorie malnutrition and compared to an oil solution of vitamin A also given parenterally. Four and twentyfour hour serum values for vitamin A content were 15 and 40 times higher following the administration of water-dispersible preparation.

#### Early Studies on Oil and Aqueous Vitamin A Preparations (7)

The period of time 1944 to 1952 was a very active study period on the oral efficacy of oil-base versus water-base vitamin A preparations for



premature infants and young children. Aqueous dispersion of vitamin A administered orally was noted to bring about a higher blood level in premature infants ( 24, 25, 26 ) or full term infants (27, 28, 29, 30 ) or young children (27, 28, 29, 30) or adults ( 27, 28 ) than did oil solutions. Fecal excretion of vitamin A was somewhat greater following oil solution ingestion than for aqueous dispersions (27) indicating that vitamin A in oil solution was less-well absorbed. In conditions affecting fat absorption or transport such as  $\alpha$ - $\beta$ -lipoproteinaemia or acanthocytosis (31 ), celiac syndrome (32, 33, 34 ), cystic fibrosis of the pancreas (27, 28, 30, 33, 35 ), obstructive jaundice (27, 28), steatorrhea ( 34, 35 ), etc., the administration of vitamin A in an aqueous vehicle was observed as markedly more effective than oil solutions. Vitamin A alcohol (retinol) and vitamin A ester in water-dispersible forms has been reported to perform equally well (36 ) in normal children; likewise, no difference in performance has been noted between the two forms in oil solution in normal children (29, 34 ).

#### Vitamin A-E Interrelationships

Even though vitamin E was discovered fifty years ago, its potential role in the practical nutrition of man and animal has been less well understood and less widely esteemed than vitamin A until the last ten years. Total chemical synthesis was achieved about 35 years ago and large volume production of dl, $\alpha$ -tocopherol and the more stable ester, dl,  $\alpha$ -tocopheryl acetate, are current realities. d, $\alpha$ -Tocopherol isolated from nature and





the chemically produced d,  $\alpha$ -tocopheryl acetate ester are also in current production.

Vitamin A absorption is impaired in vitamin E deficiency and vitamin E adequacy improves vitamin A absorption (37, 38, 39). Green (40), writing on the interrelation of vitamins A and E, cites the importance of the sparing effect of vitamin E on vitamin A under certain physiological conditions. Both vitamins A and E influence the structure and function of biological membranes according to Roels et al (38). Synergism between vitamins A and E has been reviewed by Moore (41) and Green (40).

McLaren et al (11), reports malnourished children with xerophthalmia have significantly lower serum vitamin E levels than malnourished children without ocular lesions. Low levels of vitamin E have been found in calorie-protein deficiency by Scrimshaw et al (42), in Central America by Trowell et al (43) and Tulloch and Sood (44) in Uganda, by Woodruff (45) in Lebanon, and by Rahman et al (46) in East Pakistan. McLaren et al (47) further observed that in children suffering from protein-calorie deficiency, those which recovered had a higher serum E value than those who died.

In some children with kwashiorkor in Jordan and Africa, increased hemolysis or decreased erythrocyte survival time and anemia were found which were improved by the administration of vitamin E, (48, 49).

Whitaker and Fort (50) also showed that vitamin E is a stimulant to hematopoiesis in children of Thailand. Oski and Barness (51) have demonstrated that the hemolytic anemia of premature infants responds very well to vitamin E administration and erythrocyte hemolysis decreased. The interrelationship between vitamin E and





protein is not clear. Whether a relationship exists between vitamin E deficiency and protein inadequacy in anemia in children (52) needs to be studied. It would be wise to administer a little vitamin E with the vitamin A to vitamin A deficient subjects.

Addition of vitamin A to Food Products (53, 54).

Fortification is generally considered as the addition of one or more nutrients to a food ingredient in amounts significant enough to render the food a good to superior source of the added nutrient(s) in order to minimize the appearance of disease(s). Addition of nutrients to food to compensate for nutrient losses attributable to harvesting, processing, refining, storage and/or cooking is defined as restoration. Initially enrichment was the term adopted specifically for restoring nutrients to cereal products; namely, enriched white bread, white flour, corn products, rice, pasta and cereal products. Current practice by many is to use the terms, fortification and enrichment, interchangeably. Where guidelines would be set up for selected individual foods and/or selected classes of food by an industry-nutrition-food science or government panel, to be re-evaluated every five years and modified if new data so indicate, such foods could be called "nutrified". "Nutrification" is generally considered "to make nutritious" and should be more meaningful to the consumer than the misused terms fortification and/or enrichment.

Philosophy of Adding Vitamins to Food (53)

Surveys continue to reveal signs of malnutrition in the world. It is probably a result of a changing agriculture, greater food processing, purchasing power inequities, ignorance of nutrition, social changes, changing



attitude to housekeeping, less meals consumed as a family unit, food snacking, food faddism, and the phenomenal array and number of foods in the same market places from which to choose for adequate diet preparation and consumption. Food fortification and enrichment practices, primarily turned to in times of calamities in the past, have not kept pace with the modern growth in food products and dietary habits.

Man has been nourished by consuming a variety of foods selected from the plants and animals present in this environment. While each of these foods contains some of the 50 or more nutrients required for man's nutrition, none contains all the nutrients in the proper amounts and proportions. Thus, man must consume a number of these foods daily if he is to obtain a reasonably adequate diet day by day. People usually rely upon instinct, chance and some nutrition knowledge for the selection of diets.

Food and nutrition scientists of today can assist man toward a more adequate diet (a) by combining individual foods into food products which are more balanced in nutritional value than any of the single components and (b) by restoring, enriching, and fortifying individual foods and food products with selected vitamins, minerals, protein concentrates, and amino acids so as to produce nutritious foods at relatively low cost.

The addition of nutrients to food is the most rapid, the most economical, the most flexible and the most socially acceptable method of changing the nutrient intake of a given population. It involves the least dietary changes and





and makes maximum use of native or known foods already on hand: It likewise can be applied to newly introduced and novel foods to help make them superior nutritionally, yet varied and economical. The availability of nutrients chemically synthesized helps to maintain man by freeing him from the lack or dependence of a supply of any particular natural or processed food.

#### Criteria and General Practice for Vitamin Addition(55)

To insure success of an enrichment food program, the following criteria are required: readily obtainable nutrients, low cost, undetectable changes in taste and appearance, and uniform nutrient distribution. The ideal food carriers of added vitamin A are those which the target population will purchase and consume on the basis of preference, habit and economics.

The American Medical Association Council on Foods and Nutrition and the Food and Nutrition Board of the National Academy of Sciences-National Research Council issued a joint statement ( 56 ) on fortification in 1968. The addition of nutrients to foods is endorsed when in keeping with all the following circumstances:

- A. The intake of the nutrient in question is below the desirable level in the dietary of a significant percentage of the population involved.
- B. The food carrier chosen to supply the deficient nutrient should be consumed in quantities that will make a significant contribution to the diet of the population in need.





- C. The addition of the deficient nutrient should not create an imbalance of essential nutrients.
- D. The nutrient added should be stable under proper conditions of storage and use.
- E. The nutrient should be physiologically available from the food.
- F. There should be reasonable assurance against excessive intake to a level of toxicity.

Where possible, a natural traditional association of a specific food carrier as source of the missing nutrients should also be considered; however, this is not essential. If a food low in content of the missing nutrient is present in the dietary, it might be determined if the level can be increased by fortification. Likewise, if the missing nutrients should be related metabolically to other nutrients in a food carrier, this is an additional reason for selection of that carrier. To be most effective, administered vitamin A should be given with an adequate protein intake for proper and maximum vitamin utilization. Hence, foods such as vitamin supplemented protein foods are desirable carriers. Food processing, extraction, fractionation, etc. can lower natural vitamin A content significantly.

The method of vitamin addition is frequently dependent on a food manufacturer's processing system and preferences. In many cases, the vitamin must be added before heat processing steps, e. g. , canned foods; in others, vitamin can be added after processing resulting in superior stability profiles, e. g. , flaked cereals and potato chips.



In most food development projects, the appropriate market form of the vitamin is chosen after the stage and mode of addition are decided (55). The product is laboratory prepared, simulating the anticipated commercial process and also packaged as closely as possible, in the expected manner. Samples freshly prepared and others previously stored are used to determine organoleptic effects on the food and the stability of the added vitamin. In almost all applications an input or overage above the label is required because of (a) imperfect distribution, (b) analytical error, and (c) possible degradation during processing and storage. The overages required vary with the stability of the vitamin form and the specific application. Dry stable forms of the vitamins can be premixed with appropriate overages and then conveniently added to both dry and liquid foods.

#### Foods Suitable for Vitamin A Addition and Techniques (53, 55)

The commercial forms of vitamin A produced by chemical synthesis and suitable for food fortification, either in liquid or stabilized dry form, are the acetate and palmitate esters, the palmitate being the preferred form for water-base foods. Many application problems have been solved. Margarine batch cans containing vitamin A+D<sub>2</sub>, and oil soluble color customized to supply the exact equipment and processing requirements of specific margarine manufacturers are similarly available. Rinse proof, fortified rice concentrate with vitamins B<sub>1</sub>, niacin, and iron was developed 25 years ago and now extended to vitamin A. Frozen vegetables can be fortified with vitamin A but the home





cooking procedure is critical to avoid excessive leaching into the cooking liquid. Vitamin A products can be used in baking and extruded foods. Excellent stability in white bread, tortillas, and chappaties is reported. Emulsions of vitamin A can be sprayed onto breakfast cereals, but stability is greatly dependent on the composition of the emulsion and the other ingredients in the spray solution. As a generalization, stability of vitamin A is superior in high sugar content breakfast cereals. The added sugars apparently coat the vitamin A and act as an oxygen barrier. Vitamin A can be used to fortify macaroni with only moderate losses during processing, storage, and cooking (55).

#### Edible Vegetable Oils

Vegetable oils can be fortified with vitamin A. While this is not practiced to a large extent in commerce, it is feasible if the oils are packaged in opaque or dark colored containers to eliminate the effect of light as a stimulant to oxidative processes. Edible antioxidants should also be added to protect both the vegetable fat and added vitamin A from oxidation. Inert gas replacement for the air in the headspace of the container is advisable, if practical.

#### Margarine (57)

Margarine, commonly used as a bread spread interchangeable with butter, is generally fortified with vitamins A and D to equal or exceed the average levels of these nutrients in butter. Fortification of margarine with vitamin A is now carried on in Brazil, Colombia, Costa Rica, Denmark, Great Britain, Norway, Sweden, Australia, Austria, Belgium, Canada, Chile, Finland, Germany, Greece, Israel, Italy, Japan, Mexico, the Netherlands,





Newfoundland, Peru, Philippines, Portugal, South Africa, Switzerland, Turkey, USA, USSR, and Venezuela. In about a half-dozen countries fortification of margarine with vitamin A is compulsory; however, in most countries it is a voluntary program. Where the program is voluntary, a survey should be made yearly to establish how universal the practice is and whether further legislation making the practice mandatory is required. The amounts vary between 15,000 and 50,000 IU per kg. In Germany, the standard amount is 20,000 IU per kg. In the USA, the amount is 15,000 IU per lb.

Vitamin A esters are stable in margarine without added anti-oxidants. The actual fortification of margarine with vitamin A or vitamins A and D and even E, is relatively simple. Since all are fat-soluble, a batch container can be prepared, premeasured in vitamin content to the batch size of the margarine oil tanks used in a particular margarine plant and ready to open and be used. One container of vitamins is added to one tank of oil, a simple act and an easy plant control operation. Bulk vitamin dilutions can be used, if desired, but most plant managers prefer not to leave the critical weighing of the vitamin additions to the production plant personnel. The availability of synthetic beta-carotene made it possible to both color and vitamin A enrich margarine simultaneously. A safe color,  $\beta$ -carotene can provide much of the required vitamin A activity. Available data indicate excellent stability of these concentrates in sealed containers. In the past (57) fortified margarine containing synthetic and natural A have been subjected to four types of test storage conditions: (a) Refrigerator temperature, 40°F, (b) room temperature 72-75°F, (c) alternating refrigerator and room temperature,



and (d) at 86 deg.F. In most instances the latter temperature, an extreme test, causes partial softening of the margarine.

Data on products prepared by ten manufacturers (Table 13) reveal synthetic vitamin A to be equally stable to that from natural sources. Furthermore, the data reveal that manufacturers add an overage to insure labeled vitamin content. The use of synthetic vitamin A also is greater assurance against "fishy" flavors sometimes noted with fish oil concentrates. Biological assays demonstrate full availability of the added synthetic vitamin A.

Standard type cakes and cookies, in which a commercially prepared margarine fortified with synthetic vitamin A served as the fat source, were baked in a home type electric range at an oven temperature of 375-400 deg.F. White sauce and cake frostings were also prepared to examine behavior of synthetic vitamin A during processing. Results (Table 14) affirm the findings that in biscuits, cake, and bread prepared from fortified fats it appears that 80-100 percent of the vitamin survives the baking process.

#### Peanut Butter (54)

The fortification of peanut butter with vitamin A and the restoration of the vitamin B<sub>1</sub> destroyed in roasting the raw peanuts is nutritionally sound.

While raw peanuts have a high natural vitamin B content, roasting destroys 80 to 90% of the vitamin B<sub>1</sub>. The other members of the vitamin B complex, namely, vitamin B<sub>2</sub>, vitamin B<sub>6</sub> and niacin, are not seriously affected by roasting.





Peanut butter is not only used to a certain extent as a replacement spread for butter and margarine but is also an important protein source when used in significant amounts. It can successfully be a fortified vehicle for vitamins other than vitamin A. In the commercial vitamin A fortification of peanut butter the addition should provide a claimed content of at least 15,000 IU of vitamin A per pound. This level of fortification provides 37% of the minimum daily adult requirement (MDR) per two ounces of product. Initially, an excess of vitamin A (about 25%) is added to take care of manufacturing and storage losses. Assay data on the first few runs of a particular operation will confirm whether the vitamin A overage is in the proper range. Added vitamin A is quite stable in peanut butter (Table 15).

Fortification of peanut butter can be accomplished (a) by oil dilutions of vitamin A ester mixed into the butter after grinding or (b) by premixing dry vitamin A forms with the added salt. Batch size, hermetically sealed cans containing the exact amounts of oil dilution vitamin A required to fortify a production run of peanut butter to the specification of the manufacturer can be prepared. Batch size cans are convenient because the weighing, diluting, packaging, assaying, etc., of the vitamin A concentrate is eliminated in the food plant. In the dry method, vitamin A may be readily incorporated into peanut butter by previously mixing it with the salt required. This mixture is easily prepared in tumbler-type mixers or other suitable equipment. The salt required for one pound of peanut butter should contain the amount of vitamins to be added per pound of finished product. A granular or fine granular salt as customarily employed is used.





### Milk Products (58, 59, 73, 75)

When butterfat is removed from milk the fat-soluble vitamins including vitamin A are removed. The rationale for using milk as a vehicle for the fat-soluble vitamins has been well documented. All skim milk or low-fat milk products should have vitamins A and D added and, if given to infants, should probably have vitamin C added as well. There are three principle problems involved with the addition of vitamin A to milk products, namely, taste, stability and distribution. However, by using special products prepared for milk fortification, these problems have been solved.

Liquid skim milk or partially skimmed milk (such as 2% fat) can be fortified with vitamin A in several ways. The product of choice to use is a water-dispersible stabilized beadlet, although a vitamin A solution in oil can also be used under certain circumstances. The most common methods are:

(a) Vitamin A beadlets dispersed in warm water or liquid skim milk is added to a holding tank. This is the simplest method as it is easy to weigh out the required quantity of beadlets for "x" gallons of milk in the tank.

(b) In many dairies the holding tank method is not feasible and therefore, an emulsion of the vitamin is metered directly into a fluid milk line prior to pasturization.



In the fortification of nonfat dry milk (dry skim milk powder) with vitamin A (and D or E), (a) the vitamin preparation can be dissolved in a triglyceride base such as coconut oil and blended into condensed milk prior to spraying or (b) the non-fortified nonfat dry milk can be blended with a vitamin A and D dry stabilized beadlet product in a controlled dry atmosphere to achieve a final fortified product at an anticipated stable vitamin potency when properly packaged and stored. In practice these fortification plans can be described as follows:

- A. The batch process. The required amount of dry milk powder is mixed in a batch mixer with the correct amount of water-dispersible vitamin A and D beadlets. This is the ideal method but is highly impractical for large production plants.
- B. The continuous liquid process. Liquid vitamins A and D can be introduced into the condensed milk line where sufficient agitation will assure uniformity. Directly after the evaporator is a suitable spot in most plants. To insure stability of both vitamin A and the milk flavor, it is usually necessary to dissolve the liquid vitamin in a small portion of coconut oil and inject this mixture into the line.
- C. The continuous dry process. An efficient method has been the introduction of water-dispersible vitamin A beadlets just before the instantizer, if the milk is instantized.





Problems to be aware of in the fortification of dry skim milk are uniformity of introduction of vitamin A in the continuous process, good blending in both the continuous and batch symptoms and to avoid over processing which leads to off-flavors. Stability data of added synthetic vitamin A to nonfat dry milk are excellent (Table 16). Data are also available to show that added synthetic vitamin A to dry milk is fully biologically available (Table 17). Vitamin A fortified nonfat dry milk has also been subjected to extensive flavoring testing (Table 18) and vitamin stability after reconstitution (Table 19). Packaging does influence the vitamin A stability of the dry product (Table 20). Much, if not all of the dry milk exported under the Food for Peace Program has been fortified ( 60 ). After adequate experiments had demonstrated that vitamin A is stable in nonfat dry milk, the product was fortified with 5,000 IU of vitamin A/100g, and 500 IU of vitamin D/100g. for the P.L. 480 distribution in May 1965.

Synthetic vitamin A palmitate concentrate in a coconut oil dilution has been introduced in pediatric and geriatric milk products prior to homogenizing and before the canning and sterilizing or drying operation (Table 21). Vitamin A seems to be absorbed well by the infant in diets with either the coconut or the cow's milk fat content.

In the USA, most of the bottled fluid low-fat and nonfat milk has been fortified with vitamins A and D and fortification of dry nonfat milk products has been initiated. Fortification of nonfat dry milk was approved for the





USA market in 1968. For the domestic distribution program, levels of 2,200 IU of vitamin A/100g and 440 IU of vitamin D/100g are used.

Vitamin A has been successfully added to ice cream (Table 21).

In a number of states in the USA ice cream can be made from milk products with the butterfat removed and vegetable fat substituted (mellorine).

Several states require vitamin A fortification of mellorine at a level of 8400 IU per gallon of final 10% fat product. Mellorine is usually fortified with 20,000 IU of vitamin A per gallon of mix. This provides 10,000 IU of vitamin A per gallon of frozen product, assuming a 100% overrun, and safely meets the legal requirements of 8400 IU per gallon containing 10% fat. The required vitamin level will, therefore, vary with the fat content. Dry vitamin A palmitate beadlets provide an economical and practical means of adding the vitamin A. This product dispersed in warm water is added in the mix without the necessity of homogenizing an oil solution of vitamin A into the mix.

Extensive testing has shown that vitamin A is very stable in mellorine. In the amounts used, the vitamin has no effect on flavor of the product.

Winter butter naturally low in carotene is not fortified with vitamin A. This could be accomplished with synthetic vitamin A or better still with synthetic  $\beta$ -carotene, the latter of which would also



return the proper shade of yellow color concomitant with improved vitamin A value.

Cereals and Cereal Products (61, 62, 63)

The development in recent years of stable dry forms of vitamin A of a variable range of particle size has greatly broadened the scope of foods amenable to vitamin A fortification. It is now possible to fortify a number of products which could not be fortified with liquid vitamin A preparations. Foremost among these are cereal products, such as white flour, corn or maize meal, etc.

The need for fortification of grain products with vitamin A was first suggested to the vitamin industry by UNICEF personnel ( 63 ). Vitamins B<sub>1</sub>, B<sub>2</sub>, niacin and iron are readily available in dry, fine particle forms which are easily mixed together with a carrier. The resulting homogeneous mixture (premix) then feeds well in continuous feeders. The crystals or particles of these vitamins and iron are sufficiently small, approximately 100-300 mesh, to blend well into corn meal and flour and to supply a large number of particles per pound to distribute uniformly in flour and corn meal and hence make it relatively easy to sample, assay and control the added vitamin levels.

The new small particle, dry vitamin A product, Type 250-SD introduced in corn meal and white flour in premix form, in trial runs has shown a stability in corn meal (15% moisture) of 96% retention after six months at room temperature. It has similar stability in wheat flour





containing 13.5% moisture. In flour of 11% moisture, typical retention is 90% after six months at room temperature. Stability in baking tortillas is 95% and is approximately the same in chappaties. In white bread the retention is 87% after baking and bread five days old still retains 85% of the vitamin A.

Another potential stress during milling is impact milling to destroy insect eggs. To determine if fracturing vitamin A particles by impact milling would decrease stability of the vitamin A, this product was intentionally ground in a mortar, then ground in a Waring blender and stability data obtained. The results were essentially indistinguishable from the unfractured product ( 63 ).

Vitamin A in white flour fortified with stabilized dry vitamin A premix is quite stable during storage at room temperature. The fortification of bread and flour in underdeveloped countries such as Jordan, India and Pakistan is being studied and appears feasible ( 61 ). Unfortunately, cereal-eating nations often survive on bread and rice and these, in the processed form as usually consumed, are inadequate in vitamin A (except possibly yellow corn). Assuming a vitamin premix were added to these diets, it should supply an amount per kg of diet to provide for vitamin A adequacy in the daily diet.

The vitamin A product premix was first used to fortify High Protein Wheat Flour Blend A to determine continuous feeding and mixing characteristics in a commercial run of several million pounds. Vitamin A assays





were run on daily samples. The results indicated excellent uniformity with no analytical difficulties. Because of the relatively high error, 8 to 10%, in analyzing vitamin A, it is necessary to use an overage in all food applications of vitamin A. An input of 4,700 units per pound of flour is recommended to meet the minimum USDA specification of 4,000 units (63 ).

Hence, fortification of corn meal and flour is technologically feasible. The vitamin A premix is usually added with a mechanical feeder at the rate of 1/4 ounce of premix per 100 lbs of flour. Vitamin A fortification of corn meal became a part of P.L. 480 purchase specifications December 1, 1968 and of flour May 1, 1969. The difference in date was based on the necessity to establish that vitamin A could pass through the screening or sifting operations in flour mills. The range specified was 4,000 to 6,000 IU/pound (60).

In December 1967 fortification of white bread with vitamin A, B vitamins, and iron was instituted in a new government-owned bakery in Bombay (61 ). Since that time four more bakeries have been constructed, and still more are to be built. Vitamin A is an ingredient of the fortification mixture used in all the present plants, and the same formula will be used in the plants to be built in the near future. The level of vitamin A is 8,000 IU per kilogram of bread. A program is about to get under way involving the fortification of both dark and light wheat flours with vitamin A as well as B vitamins, iron, and calcium. This program is being started in the largest flour mills in major population centers, and was to be extended

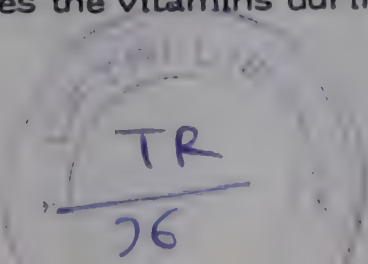


to nearly 200 flour mills. The estimated total quantity of wheat flour involved in 1968 was around 330,000 tons.

Rice, without the husk, is the staple food of well over half the world's population (64). Like wheat, rice grains consist of a starchy kernel surrounded by a seed coat and enclosed within an outer fibrous husk. The bran (coat) and germ, containing the majority of the vitamins of the B complex, is removed by polishing in special mills to obtain white rice. Like white flour, polished rice mainly consists of starch and no longer contains these essential vitamins. The solution to this dietary problem inevitably involves replacing in suitable manner the vitamins 'lost' from the rice. Several methods have been developed for rice enrichment (61).

#### Roche Method A

A rice premix developed over the last twenty-five years, which is added to 'normal' rice, has proved technically practicable. Rice grains are allowed to absorb a solution containing vitamins B<sub>1</sub>, B<sub>2</sub> and niacin, whereby they take up several times their original vitamin content. The grains are then given a coating to protect the vitamins as a century-old practice exists to wash the rice with cold water before cooking. The fortified grains are then added to the rice in an appropriate ratio (for example 1:200), so that the finished product contains the same amount of vitamins as unpolished rice. Dry vitamin A maybe added prior to or during the coating operation. The coating breaks down and releases the vitamins during the cooking process.







### Roche Method B

Unlike the customary procedure, a new method not based on the use of rice itself, but on a rice-like paste product as a vitamin carrier has been developed. It has the advantage that other substances such as vitamin A and amino acids can readily be incorporated into the premix. In the normal course of manufacturing dough for food pastes (macaroni, noodles, etc.) the necessary quantities of vitamins can be dissolved (B complex) or dispersed (vitamin A powder) in the water used, or can be added directly to the flour in dry form. The dough is then processed further as for food pastes. The simplest means of obtaining rice-like grains is to produce a type of spaghetti of the appropriate diameter, which is then cut to the length of the rice grains. If desired, these particles may be shaped to resemble rice still more closely. Otherwise, rice-like grains can be produced directly from the press by using a suitable die. The concentration of vitamins can be chosen so that each 'grain' contains 200 times that of the unpolished rice, and the premix would then be added to polished rice in a proportion of 1:200. According to the nature of the deficiency in the particular country, other ingredients such as minerals, amino acids, etc., could be incorporated into the same premix or alternatively, more than one premix could be prepared and added to the rice as required. If, for dietary, legal, commercial or consumer preference reasons, it is undesirable to use wheat, the premix could also be produced using, for example, rice meal.





Vitamin A in dry form can be used in either type of premix kernels.

Considering the cold water washing of rice before cooking and some losses during cooking a vitamin A excess of about 30 to 40% should probably

be made in the premix addition to white rice. Brooke ( 61 ) reminds us that effective fortification of rice can only be accomplished when the consumer can be trained to cook rice in just enough water to bring the rice to the optimum consistency. Cooking in several volumes of water and pouring off the excess after cooking results in heavy losses of added vitamins.

#### Potato Products (65)

The availability of stabilized, finely particulated dry vitamin A has made it possible to fortify dry potato granules in addition to cereal products. While the white potato does not naturally contain the carotenoid vitamin A precursors ( $\beta$ -carotene) as the sweet potato, a high tonnage of dry white potato products are produced. Early experimentation of adding a dry vitamin A source to dehydrated potato flakes has indicated, when properly processed and packaged that the added vitamin is stable (Table 22).

Highly nutritious potato granules and flakes fortified with vitamins A and C and, in some instances, B<sub>1</sub>, B<sub>2</sub> and niacin are easily prepared.

#### POTATO GRANULES

Suggested Levels Per Serving — Manufacturing Overages

| <u>Vitamin</u> | <u>Amount of Vitamins</u> | <u>Overage</u> |
|----------------|---------------------------|----------------|
| A              | 1000 to 2000 USP Units    | 25%            |
| C              | 15 to 30 milligrams       | 20%            |
| B <sub>1</sub> | 0.25 to 0.50 milligram    | 25%            |
| B <sub>2</sub> | 0.12 to 0.24 milligram    | 20%            |
| Niacin         | 2.5 to 5.0 milligrams     | none           |



The moisture content of the granules should be kept at, or below, 5% to 6% to minimize vitamin losses and development of rancidity. Nitrogen packing will improve vitamin stability and will usually extend product shelf life. Potato granules are easily fortified by dry blending techniques as follows:

- A. Prepare a premix of the vitamins with potato granules in a ribbon mixer, a twin shell blender, or any other blender of dry materials.

In preparing the premix, blend the vitamins with at least 1% of the total weight of the batch. For example, if 1000 pounds are to be fortified, prepare the premix with 10 pounds of granules. Where possible, prepare the premix with 2 to 3% of the total weight of the batch to get faster distribution of the vitamins in the main portion of the batch.

A stock supply of premix may be prepared for several days' production. When prepared, the stock premix should be stored in well-closed, polyethylene-lined fiber drums in a dry, cool place. A silica gel bag should be packed with the premix for moisture control.





- B. The vitamin premix should be added slowly to the remainder of the batch as the blender is operating. Twenty to thirty minutes of dry blending should give good vitamin distribution. To establish the best mixing procedure, samples should be taken for assay at ten minute intervals. The mix is uniform when good checks are obtained on two consecutive samples. The minimum mixing time can then be standardized at the time required to obtain the first of these samples.
- C. Where continuous operations are in process a mechanical feeder is used to uniformly introduce the premix to the flow of potato granules or flakes.

#### Fruit Products (66)

Bunnell has called attention to the large variation in the amount of provitamin A supplied by different fruits, as well as a considerable variation in the provitamin A content of any single fruit. Considering the variability in the natural vitamin A levels of fruits, vegetables, and butter, Bunnell points out that margarine, which is standardized to provide a definite amount of vitamin A, is the only reliable and consistent source of this vitamin among the food groups mentioned. Bunnell suggests that in fortification of fruit juices to a specific level with vitamin A, beta-carotene be employed to impart the type and depth of color desired, and that the prescribed level of activity be attained through supplementation with synthetic vitamin A in water dispersible form.





### Confections (54)

Confections have been fortified with synthetic vitamin A. A series of trials were set up fortifying confection products with synthetic vitamin A. The data (Table 21) demonstrate that fairly good stability can be achieved. An added overage of 15-33 percent beyond the claimed vitamin A value may be necessary, depending on expected storage conditions. The following were studied:

A. Caramels were prepared from a basic formula consisting of corn syrup, hydrogenated coconut oil, sugar, milk solids, and lecithin. Vitamin A palmitate and vitamin D were introduced in the coconut oil ingredient prior to processing. The entire mixture was cooked for approximately 18 min. to a temperature of 240°F.

B. Chocolate ration bars, produced in commercial equipment, were fortified with vitamin A and other vitamins on the basis of one ounce of chocolate supplying 2,500 IU vitamin A. Vitamin A acetate or palmitate was carefully mixed into small portions of the chocolate-cocoa butter base. The ingredients of the bar, namely chocolate liquor, cocoa butter, sugar, salt, milk, vanillin, and lecithin, were mixed together, passed through the roll refining mill and the melangeur, and molded into bars.

C. Chocolate fudge was prepared according to commercial procedure from heavy cream, sugar, corn syrup, and evaporated milk. All ingredients were mixed together and heated to 240°F. The candy mass was cooled to 150°F, at which point melted shortening containing vitamin A was incorporated. Shortly thereafter the mixture was poured.



D. Molasses taffy has been successfully fortified with synthetic vitamin A.

The basic candy was made from corn syrup, sugar, condensed milk, butter, water, and molasses. The candy was cooked to 250°F and poured onto a marble slab. Vitamin A palmitate concentrate and vitamin D were folded in on the slab. At a temperature of 130°F the candy was pulled, then cut and wrapped.

#### Tea Products (67)

Surveys covering several of the Indian states show that millions of young children are given 1-2 cups of tea per day. A food habits survey conducted in the Western India states of Gujarat and Maharashtra by the Protein Food Association (1969) revealed that 87% of the villagers in Gujarat give tea to their children, including 84% of those in the lowest income families. Thus it appears that tea comes close to being the ideal vehicle for conveying vitamin A to millions of Indians of all ages whose diet is seriously deficient in the vitamin. The idea of using tea as a vehicle for conveying meaningful quantities of vitamin A to an entire population was conceived at the New Delhi Mission of the Agency for International Development as early as 1967.

The technology involved in fortification of dried tea with vitamin A is non-existent in the literature. Tea fortunately contains many natural antioxidants such as catechol, epicatechol, and gallic acid, to name a few. Many of the phenols and polyphenols function as antioxidants and should also be an aid to the stability of vitamin A in tea.





Procedures to fortify tea were investigated in laboratory studies (67):

- A. Tea Dust is consumed by 65% of the population in India. It is not an inferior product; the term dust merely refers to the fact that the product consists of the smallest tea particles. As a rule, it is quick brewing and produces a darker, stronger brew. Dusts are also used for blending with small or broken leaves and are in demand. Dusts are not to be confused with "fluff" and "chalk," which are "tea waste" not included in dusts. Tea dust was fortified by dry mixing the dust with fine powdered vitamin A palmitate 250 SD. This is a dry, stabilized, fine powder originally developed for flour. It contains 250,000 IU of vitamin A per gram. The fortified tea dust retained 85% of the vitamin A activity after storage for 1 year at room temperature (Table 23).
- B. Tea Leaves were fortified using emulsions of vitamin A palmitate and acetate. The emulsions were made by homogenizing the vitamin A into thick acacia or dextrin solutions with a homorod at 500,000 IU/g. These emulsions are 100% stable for 1 year at 24°C but are too concentrated to be used directly. Consequently, they were diluted (in water, dextrin solutions, or sugar solutions) and then sprayed onto the tea leaves but only vitamin A emulsions diluted in 50% sucrose solution and sprayed on tea resulted in satisfactory stability. It was also established that special





water-soluble oil solutions could be diluted in sucrose solutions and applied to tea with reasonable stability. (Table 23)

Since the vitamin A must survive brewing, the retention of vitamin A was measured after brewing. The initial target was a 5-min boiling time, since this does produce a drinkable tea. However, a review of cooking practices revealed that tea is often left on the fire for as long as an hour.

Comparative recovery from brewed tea (Table 24) indicates that boiling is detrimental to the acetate form as well as to the special water-soluble oil form of vitamin A palmitate<sup>1</sup>. However, vitamin A palmitate applied in both the powdered form and emulsion form showed 100% retention after 1 hr of boiling.

Therefore, laboratory testing proved that tea dust or tea leaves can be readily fortified with vitamin A and that the vitamin A will be stable even during a 1-hr cooking period.

Trials on a commercial scale in a tea factory in South India have demonstrated the feasibility of fortifying large quantities of tea during processing in individual factories or in blending plants. The vitamin A may be applied either as a dry powder blended into the finished tea or as a diluted water-dispersible emulsion sprayed onto the hot tea as it emerges from the drying chamber.



The studies thus far have aimed at fortifying dry tea to a level of 125 IU of vitamin A per gram. The usual adult cup holding 150 ml is brewed from 3 g of dry tea. Thus a cup of tea would supply 375 IU of vitamin A (67).

### Sugar (68)

Although the consumption of sugar in the U. S. has not increased in the past 20 years, the per capita consumption is twice the world average. Adolescents and young adults consume large quantities of carbohydrates, especially sucrose. In response to cultural and technological demands, sugar is now so purified that many sugar-rich foods are nearly devoid of minerals and vitamins, and contribute nothing but calories to the diet (69).

Consideration should be given to the enrichment of sugar and sugar-rich food products with thiamine and niacin to make sugar metabolically independent. Consideration should also be given to the supplementation of sugar and sugar-rich food products with other ingredients to thus combat the development of dental decay which pure sugar promotes. If enrichment of sugar-containing foods is advisable for the U. S. and the western hemisphere, it is certainly even more important in the less technically developed countries of the world where the consumption of sugar-containing foods is increasing. A technology has been developed for the addition of vitamin A to certain sugars.

A project was initiated at INCAP with the cooperation of Hoffmann-LaRoche Inc. in 1969 to test and implement the fortification of a (68) suitable vehicle with vitamin A. The following characteristics of a suitable vehicle were examined: (a) universal consumption by the population, (b) little variation in consumption per capita from person to person and from day to day, (c) supplementation resulting in an





unappreciable change of organoleptic characteristic and (d) the cost and nature of the vehicle to make the process of supplementation economically feasible at an industrial scale of operation. Based on these, refined sugar was selected. Sugar has all four characteristics. Besides, it is produced centrally in only a few large technically well equipped factories, where the addition of vitamin A could be carefully controlled (68).

The average consumption of white sugar by the population of Central American countries was determined from data collected during the regional nutrition surveys carried out in 1965-67 by the INCAP-OIR-National Governments cooperative effort of eating habits. Based on the retinol potency of the vitamin A palmitate preparation selected, the amount to be added to the vehicle was chosen so that the average per capita per day consumption would provide the FAO-WHO Recommended Allowance for an adult man, namely, 750 mcg of retinol or 2500 IU of vitamin A.

Of several vitamin A concentrate types on the market, retinyl palmitate, type 250 SD, manufactured by Roche was chosen. Its potency is 250,000 IU per gram and is a water-miscible powder. Its color is pale yellow and has a slight characteristic odor. Since the vitamin A concentrate to be used contains 75,000 mcg of retinol or 250,000 IU per gram, 36g sugar, the average per capita consumption per day should contain 0.01 grams of the vitamin A concentrate which, when expressed in pounds (460g) and quintales (100 pounds), is 0.128g per pound or 12.8g per quintal. A sugar so prepared has the following potency: 1g=20.8 mcg of pure retinol or 69.5 IU of vitamin A (68).





In the process of sugar fortification, a mixture is prepared of one part of Type 250-SD uniformly blended with nine parts of "El Salto" sugar, a finely crystalline sucrose, to yield the vitamin A-sugar premix (1+9). The uniform premix, in turn, is placed in a suitable micro-mixer feeder which introduces the premix into the flow of regular sugar, followed by mixing to yield vitamin A fortified sugar (Figure VII). The feeder was placed at the end of the production line of the factory, after the drum-dryer. The complete system consists of: (a) a funnel which holds the fortifying material, (b) the mixer-feeder which is located right under the funnel, (c) a distribution cone which breaks the stream of fortifying material coming out of the feeder and spreads it along the width of the sugar stream. The energy for the operation of the micro-feeder-mixer is furnished by a motor which also controls the operation of the sugar elevator-belt. This insures even distribution of the vitamin A premix over the sugar stream. The amount of premix to be added by the synchronization of the feeder and sugar flow regulation of the mixer-feeder is set at the rate of 13 g of Type 250-SD concentrate or 130 g of vitamin A-sugar premix per quintal of sugar. The premix rate of flow must be synchronized to the rate of flow of the sugar to be fortified at all times followed by adequate mixing. The vitamin A fortified sugar is packed in regular cloth sacks, stored and handled in the same manner as regular sugar (68).

Lots of vitamin A fortified sugar were prepared at the specified concentration mentioned above and were submitted for color and taste tests. Samples of pure sugar and fortified sugar were first submitted for visual comparison by 30 individual panelists. The samples were indistinguishable from each



other. Then a triangular taste test followed, conducted on foods sweetened with either regular or vitamin A fortified sugar. The sugar used was locally produced white sugar. The level of fortification was the standard 69 IU of vitamin A per gram of sugar. Each panel was formed by ten members, each performing three individual observations giving a total number of thirty comparisons. The foods were prepared as usually consumed by the local population. The amount of sugar added can be considered "customary". The results were considered encouraging (Table 25). Although the flavor was detected by a significant number of panel members for some foods, the preferences were randomly distributed, and, furthermore none found the flavor to be unacceptable (68).

Evidence from the biological experiments with animals indicates that vitamin A fortified sugar which has been kept for three months under warehouse storage conditions, at temperatures ranging from 40° to 20°C, had not lost potency significantly, as judged by its effect on the regeneration of liver reserves in vitamin A depleted rats. These stability studies were confirmed by chemical analyses.

Two samples from sacks of sugar fortified at "El Salto" on May 20, 1970, with an estimated concentration of 20.8 mcg/g gave on October 9 an average of 24 mcg/g (115% of the theoretical value). Although suggesting somewhat uneven distribution, this result indicates very satisfactory stability after nearly five months. Another sack of sugar, fortified at the laboratory on April 9, at a somewhat lower concentration than the industrial, gave the following





## results:

|            | Retinol (mcg/g) |
|------------|-----------------|
| April 9    | 13.0            |
| June 3     | 15.2            |
| June 5     | 14.9            |
| October 14 | 13.1            |

This sugar was kept for six months on storage in a regular cloth sack from "El Salto" in Guatemala City. It was moved every week as one would during transport and handling. Most of the storage period corresponded with the heavy rainy season. As mentioned in a different section of this report, the length of time that sugar lasts in the market is a maximum of six months (68).

The suitability of vitamin A fortified sugar was tried in the preparation of "Lemon Crush" by "Fabrica de Bebidas Gaseosas Salvavidas, S. A.". Through the cooperation of this Guatemalan company, specialists in the industrial production of bottled refreshments, 2,800 bottles of a clear lemon-flavored sparkling water were prepared. The product obtained was very satisfactory, according to the standards for this beverage as stated in a report from the company: "a product was obtained, similar in quality to the standard product of the company, slight turbidity was noticed". The turbidimetry relative readings were:

|                       |           |
|-----------------------|-----------|
| Distilled water       | 0 units   |
| Lemon Crush           | 48 units  |
| Lemon Crush fortified | 130 units |

The turbidity does not seem objectionable (68).





A project was carried out to study acceptability of industrially produced fortified sugar when made available to a rural community in Guatemala through the most regular natural channel. The distribution of the sugar was carried out, therefore, through the town stores, without any knowledge of the members of the community. Fortified sugar was bought and consumed almost exclusively. A nearby village of very similar ecological and socio-economic characteristics, served as a "control". This project lasted approximately six months. The results indicated complete acceptability of the product. No characteristics developed in the fortified sugar to make it appreciably different from the ordinary sugar.

A question had been raised about the fact that infants being breast-fed do not ordinarily consume sugar and, therefore, they would not be getting a direct benefit from this program. Against this, however, there is the hypothesis that the lactating mother consuming fortified sugar would produce a milk richer in retinol. Previous INCAP studies have shown that rural low socio-economic women in Guatemala produce breast-milk with a very low vitamin A concentration (68).



To test this, milk from lactating women in both experimental and control villages were analyzed for retinol (Table 26). It was clear that breast-milk from mothers in the town consuming fortified sugar had significantly more vitamin A than that from mothers in the control town. Infants, therefore, would be getting their share even when exclusively breast-fed.

Arroyave (68) points out that there appears to be no danger of potential vitamin A toxicity in the ingestion of fortified sugar by the population. It appears that no major problems will be encountered in the implementation of this important public health measure at the national or regional level. In two member countries of INCAP, namely Guatemala and El Salvador, arrangements are well advanced to take this pioneering step. Specifically, the form of financing the process is being discussed at the government level with the participation of the sugar manufacturing sector and INCAP.

Fortified beet or cane sugar is now available for use in selected foods by another processor. A combination of coated and uncoated vitamins and minerals, plus selected amino acids is added to sugar, blended in stainless steel blenders equipped with intensifier bars for short periods of time to prevent damage to nutrient coatings. Dry sugar products resulting are nearly identical in appearance to conventional sugar.

Surface active compounds and anti-caking compounds are used to insure good flow qualities, and to reduce or virtually eliminate vitamin flavors from the final product. Nutrients considered most desirable include vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, niacin, vitamins C, D, E and A, lysine, phenylalanine, alanine, leucine, tryptophan, methionine, iodine, magnesium, potassium, iron and others ( a total of 13).





The process may be licensed to sugar refiners. The sugar also is available from Buckeye Sugars Co., Ottawa, Ohio. The fortifying principle was developed with the Dextra Corp. of Miami, Fla. in collaboration with Hoffmann La-Roche. Information is available from the patent holder, the Magro Co., of Cincinnati, Ohio.

### SALT (70)

Under certain circumstances, salt could be a vitamin A carrier. Salt utilization in India is widespread. Everyone uses salt, and salt production is relatively centralized. Nutritionally, salt can be an attractive carrier because it is used consistently throughout the year by all Indians, urban and rural, rich and poor, vegetarian and non-vegetarian.

Salt as a carrier for nutrients is not a new concept. Today, in many countries salt is iodized to combat goiter. In India, salt produced for the sub-Himalayan goiter belt must, by law, be iodized. This program has reduced incidence of goiter in children in Kangra Valley (Himachal Pradesh) from 38% in 1956 to 3% currently. It was this concept and the results which led, in the last few years, to thought and discussion of extending the salt fortification principle beyond iodization. Other nutrients considered have been, calcium, iron and lysine.

If vitamin A were to be added, it would have to be dry vitamin A palmitate Type 250-SD, a fine particle form of vitamin A prepared in premix form and a physically stable form and added to the salt proper with the aid of a feeder mechanism as in the case of sugar.

Salt lends itself to a fortification technique in the conventional dry-mixing or spray-mixing in a screw conveyor located at the bagging site. This is the technique which has been used for iodization at India's large public sector salt works.





Low-Cost Foods (71)

According to Weisberg ( 71) experience in developing and marketing foods in developing regions of the world indicates that the private food industry sector is a primary potential source for providing relatively low-cost protein foods. Technology exists in most instances for the incorporation of vitamin A. Although the large-scale efforts of the U. S. government agencies AID and USDA in donating food blends of high nutritive value to developing countries have been effective, these are stop-gap measures which must be supplanted through the marketing by the private food industry of nutritious foods at a viable price. Such private food enterprises have the potential to:

- (a) create markets for desirable crops, such as legumes and oilseeds;
- (b) provide employment and therefore income for many people, (c) create markets for food processing equipment and packaging supplies,
- (d) provide tax money for government functions and (e) provide continuity of supply of good foods at reasonable cost.

A well managed, profitable business can have a long life whereas food donations depend on the ever-changing climate of government intentions and self-interest. Each situation should include: (a) motivating factor, (b) capital, purchasing power and other phases of economics, (c) cooperation and role of government (d) manufacturing resources, (e) food technology, related chemical and engineering skills and available personnel, (f) availability of ingredients, (g), packaging materials and equipment and (h) distribution channels.

Some of the more successful enterprises ( 71) are based on improving an already accepted food, such as an old product like soy milk, by extending the useful shelf life, improving the flavor, and providing safe and convenient packaging. Vitasoy in Hong Kong, for example, is test marketing 3 "cheese" spreads made of soy protein and is developing textured protein foods, for which it feels there is a promising future. Textured Protein Foods, usually based on soy products are emerging in the U.S. as beef, chicken, pork and bacon-like foods. In due time they should find a major place among second





generation foods in developing countries and offers an opportunity to the U. S. food industry. Reasonably satisfactory organoleptic properties can be built into pasta products such as a modified macaroni product, as shown by General Foods Corporation's experience with consumer acceptance in developing countries. Crackers and cookies can also be fortified with needed nutrients, and such low-moisture products, if sanitarily packaged, have a long shelf life.

Some case histories of successful low-cost protein foods are given below. Their success or lack of it has been evaluated by Weisberg (71).

(1) INCAPARINA was developed by the Institute for Nutrition in Central American and Panama. Its history is worth noting because it is a record of some moderate successes and some failures.

The product is a bland, reconstitutable powder consisting of corn flour, cottonseed flour, protein concentrate, vitamins and minerals. Production in Guatemala is at the rate of some 2.5 million lb/yr. It's produced and marketed in Colombia by the Quaker Oats Company and is largely sold as an institutional product, which is repacked by the local purchasers in 1/2-kg bags. In 1961, Productos Alimenticios attempted production and marketing of Incaparina in El Salvador. There was good support from the Health Ministry, the company was qualified in respect to suitable equipment and technology, and extensive advertising was used. Nevertheless, here, the new product failed because the organoleptic properties were unacceptable to the native population. An American company attempted production of Incaparina in Brazil in 1966. The product was not successful for a variety of reasons.

(2) Golden Elbow Macaroni is a pasta product currently being franchised in Brazil by General Foods Corporation. It contains wheat flour, corn flour, defatted food-grade soy flour, minerals, and vitamins. Consumer testing in Brazil and Peru for 4 months showed good acceptance.





(3) Modern Bread is a successful fortified bread produced in India under government sponsorship. It is made from flour fortified with vitamins, minerals, and lysine. Current production is about 50 million 400-g loaves/yr, and projected plant capacity is 100 million loaves/yr. Bakeries are established in Ahmedabad, Bombay, Cochin, Delhi and Madras and under construction in Bangalore, Calcutta, Hyderabad and Kanpur.

(4) Miltone is a sterilized milk beverage sold in bottles in India. Developed by the Central Food Technological Research Institute, the product is prepared by mixing reconstituted (or fresh, undried) peanut protein isolate and hydrolyzed starch syrup with animal milk, minerals and vitamins. The nonfat solids content is adjusted to 2%, and the mixture is homogenized and pasteurized to yield a drink that has a protein, fat, carbohydrate and vitamin content equivalent to that of animal milk. The surplus of essential amino acids in the animal milk compensates for their deficiency in the peanut protein.

(5) Nutresco is a powdered product which is reconstituted with water and steeped for 20 hr to ferment. It has a mild lemon flavor and consists of pre-cooked corn meal, malted millet, malted sorghum, soybean meal, groundnut meal, fish protein, and skim milk powder. Ninety-five percent of the ingredients are of in-country origin. This product has been produced in Salisbury, Rhodesia and marketed in Rhodesia, Angola, Malawi, Zambia, South Africa, and Mozambique. It has been an item of commerce since 1964. Production ranges between 2.5 and 5 million lb/yr. It is one of the least expensive food supplements of its kind, selling in the range of 12.6-15.6 cents/lb, depending on the amount purchased.

(6) Nutrovite is a reconstitutible gravy powder which serves as a meat-like gravy. Made by the Nutritional Research and Food Products (Pvt.) Ltd. of Salisbury, Rhodesia, the product consists of groundnut, soybean, white flour, food yeast, and fish protein and comes in 4 flavors. It is mixed with cold water, brought to a boil, and simmered for 10 min. It is used as a meat substitute which is served as a gravy with the usual cereal (corn) in Africa. This





product is produced at the rate of 12 million lb/yr and is accepted by all age groups, beginning at age 3 mo. It has been marketed for 7 yr in Rhodesia, Mozambique and Angola.

(7) Pronutro is a powder containing soy, corn, groundnuts, skim milk, wheat germ, fish flour, food yeast and bone meal, with iron salt added to be reconstituted as a soup, beverage, or cereal, simply by adding hot water and sugar. It is produced by Hind Brothers in Durban, South Africa, at the rate of 100 tons/wk, or approximately 10 million lb/yr. It has been accepted in the marketplace for 8 yr and is sold in South Africa, Rhodesia, Mozambique, Lesotho, Mauritania, Malawi, and Swaziland. It has achieved a 40% penetration in trading stores in rural areas, which is a remarkable achievement.

(8) Protone is a high-protein powder blend of animal and vegetable protein products fortified with vitamins and minerals. It is reconstituted with cold water and boiled for 10 seconds and is used as a soup or gravy. Primarily sold for institutional use, the product is made by Sungold Products (Pty) Ltd., Johannesburg, South Africa, and is used in South Africa, Lesotho, Swaziland, Botswana, Congo, Malawi, and India. Production is at the rate of approximately 6 million lb/yr.

(9) Puma is a soft drink developed by Monsanto and franchised to D'Aguiar Brothers (DIH) Ltd., Georgetown, Guyana. It contains soy protein, vitamins, sugar and water is packaged in returnable bottles and in cans. It is sold in all areas (urban, village, rural) and has been accepted because of desirable flavor, ready availability, and moderate price.

(10) SACI is a sterilized product made with soybeans, cocoa, sugar, vitamins and water. It is produced in caramel and chocolate flavors by Coca-Cola Refrescos S.A. in Rio de Janeiro.



(11) SAMSON is a translucent, red-colored, non-carbonated soft drink being marketed in Paramaribo, Surinam. A product of the Coca-Cola Company, it is bottled in 7-oz returnable glass bottles by I. Fernandes and Son N.V. The product has a fruit punch flavor and contains 7 essential vitamins and 2% milk-derived protein.

(12) SAM YANG Noodles, a Seoul, Korea, company, has been very successful in producing and promoting instant fried noodles provided with a variety of reconstitutible dry sauces. The company, which has expanded production 11 times since 1963 is planning to use soy protein isolate to fortify the flour from which the noodles are made.

(13) VITABEAN is a sterilized soybean milk beverage made in Singapore by Heo Hiap Seng Ltd. It is packed in a non-returnable tetra-pak paper container lined with aluminum foil for Malaysia and in glass for Singapore. The product has a shelf life of 6 mo and is one of the least expensive products of its kind. It represents the application of dairy technology to soy milk processing. Approximately 13 million 10-fl oz containers are produced per yr and sold in Singapore and Malaysia.

(14) VITAMILK, made in Bangkok, Thailand, is a bottled, sterilized milky beverage consisting of soy milk, whole cow's milk, and vitamins. Plant capacity is 120 million bottles/yr. The product retails at 5 cents/bottle and wholesales at 3.1 cents/bottle.

(15) VITASOY is a beverage made from soybean extract fortified with vitamin A, the key B vitamins, and sugar. It is an unmistakable success in Hong Kong, where it has been marketed for 30 yr. Production at present is at the rate of over 100 million 6 1/2- and 8 oz bottles/yr and sales have recently been exceeding the sales of conventional soft drinks such as Coca-Cola.





### Nutricube<sup>R</sup>, a Dietary Supplement

There are situations and conditions where "nutrified" foods cannot prevail because of problems (a) in adding nutrients to the native grown and home prepared foods, (b) in the distribution of the "nutrified" foods to an isolated target population segment or (c) other unforeseen problems. It may, however, be possible to take a "cube" or wafer of the critical nutrients to the population segment and have it used as a sort of "instant nutrification" or "do-it-yourself" concept. Vitamin A can be an important ingredient of the "cube" concept.

Nutricube is a special dietary supplement which by custom-formulation conveys to target populations only the necessary quantities of vitamins, minerals, and/or amino acids that are not supplied in native foods at levels needed to meet dietary requirements.

Nutricube provides the designated micronutrients in a variety of convenient-to-use forms:

- (a) Beverage Granules - are mixed with water to provide a highly acceptable flavored drink.
- (b) Chewable Tablets - are eaten as is. Tablets are formulated to provide those flavors most acceptable to local consumers.
- (c) Pot Cubes - are added to the native foods in the cooking pot just before the food is served.

Nutricube "breaks the cycle" of micronutrient malnutrition immediately and economically because:

- (a) Micronutrient formulations are based on the specific needs of the consumer.





Only those micronutrients needed to supplement the dietary intake are provided.

(b) The specific micronutrients are provided easily and conveniently to consumers eating a wide variety of diets.

(c) Nutricube product forms are designed to conform to the consumer's life style. No significant change in eating or cultural attitudes is necessary.

(d) Few instructions are needed for proper use.

(e) No interference with socio-economic programs is necessary.

Nutricubes succeed. Nutricube Beverage Granules were given to school children in New Orleans, Louisiana, USA, to correct micronutrient deficiencies revealed by the National Nutritional Survey. Biochemical tests demonstrated the success of Nutricubes in providing biologically available iron, folic acid, and amino acids, which overcame the deficiencies as well as improvement in behavioral testing. Success of the program was attributed to the ease and simplicity with which the Nutricube "Beverage" was prepared and accepted by the children.

#### Cost Considerations

Previous studies have been made on the suitability of synthetic vitamin A as an economical source of nutrition in the dietary of man. A linear programming exercise (62) has been carried out by Indian scientists with the help of a computer to evolve a least-cost program for meeting the deficiencies of protein, iron and vitamin A. This exercise has shown that for meeting vitamin A deficiency in India, the cheapest method is to draw upon synthetic vitamin A. The cost of 82,222 billion international units (providing about 2,500 IU per person), the



amount needed to meet the deficiency, would be about 8 million dollars. If vegetables alone were used to meet this deficiency, it would take 6 million tons - three times as much as produced at present - and the cost would be 560 million dollars, which is 70 times the cost of synthetic vitamin A. If fruits alone were used, it would take 31.62 million tons - more than twice present production - and the cost would be 1,000 times the cost of the synthetic vitamin A. It should be pointed out that the above estimates were based on a synthetic vitamin A price level several times as high as in the United States or Europe.

Price quotations on vitamin A manufactured by chemical synthesis can be obtained quickly from producers of these products in the quantity desired. Since a variety of market forms are available, there is no single price for all forms, the price varying with type and quantity. Where vitamin A is merely diluted with oil, to provide a standard vitamin A potency per liquid product, prices are usually more economical than for physically and chemically stabilized products in dry form or for parenteral solutions. Higher prices are necessary to compensate for the added technology, processing and materials incorporated into these products. Likewise, since vitamin A is produced and sold in various parts of the world, recognition should be taken of this and when economics of vitamin A are involved, price information for the country wherein purchasing and product development are anticipated, should be used.





### Massive Dosing Forms

Bulk vitamin A must be put in some application form suitable for the intended use. If the emergency route of massive dosing of deficient children or adults two or three times a year is pursued, the following forms of administration in order of increasing cost, can be listed with their advantages and disadvantages.

| Product                                                                                                     | Advantages                                                                                                                     | Disadvantages                                                                                                                                                                                          |
|-------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Dark glass vial or bottle of oil solution of vitamin A (or A plus E) with calibrated dropper closure.    | 1. Most <u>economical</u> form<br>2. Good stability performance in closed vials<br>3. Drops easily placed on tongue of subject | 1. Not a unit dose<br>2. Amount used must be given by instruction<br>3. Possibility of vial contamination by frequent removal and return of dropper closure<br>4. Possible glass breakage or spillage. |
| 2. Bulk container of (water dispersible) dry vitamin A (or A plus E) with volume measuring device supplied. | 1. Good stability performance in closed containers<br>2. Water dispersible vitamin form                                        | 1. Not a unit dose<br>2. Amount administered could be subject to error<br>3. Preparation needed by staff in administration                                                                             |





| Product                                                                                                                          | Advantages                                                                                                                     | Disadvantages                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2. (cont. ) Product would be dispersed into some flavored beverage for consumption.                                              | 3. Allows for some flexibility in administration<br>4. In non-glass containers, no breakage should occur.                      |                                                                                                                                                                                                                  |
| 3. Chewable tablet made with (water dispersible) dry vitamin A (or A plus E) with added sweetener and flavors.                   | 1. Fairly good stability<br>2. Water dispersible vitamin form<br>3. Is a unit dose<br>4. Is quickly chewed and/or swallowed    | 1. Without teeth, child would have difficulty in consuming.<br>2. Tablet would have to be crushed for young infants<br>3. Because of the volume of excipients, sweetener and flavor, tablet size is fairly large |
| 4. Two piece hard shell capsule containing (water-dispersible) dry vitamin A (or A plus E) with or without sweetener and flavor. | 1. Good stability in sealed capsules<br>2. Water-dispersible vitamin form<br>3. Is a unit dose<br>4. Can be swallowed (cont. ) | 1. For children too young to swallow capsule, contents would have to be given in other ways.                                                                                                                     |



| Product                                                                                                                                                                             | Advantages                                                                                                                                                                                                                                                   | Disadvantages                                                                                                                                                                                                                   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                     | or (opened) contents<br>consumed alone or with<br>food.                                                                                                                                                                                                      |                                                                                                                                                                                                                                 |
| 5. One piece soft gelatin capsule<br>with a nubble or nipple<br>containing oily vitamin A<br>(or A plus E).                                                                         | 1. Excellent stability in<br>sealed capsule<br>2. Is a unit dose<br>3. Can be swallowed<br>or nubble cut off and<br>contents squeezed<br>out onto tongue                                                                                                     | 1. If stored in hot humid<br>atmosphere, capsules<br>may stick together<br>2. Should use small<br>containers with good<br>seals against moisture<br>to minimize this<br>tendency.                                               |
| 6. Glass ampule of vitamin A<br>(or A plus E) in aqueous<br>base composition for<br>intramuscular administra-<br>tion. This form is for<br>the advanced stages of<br>xerophthalmia. | 1. Good stability in<br>sealed ampules<br>2. Is a unit dose<br>3. Is water-dispersible,<br>the most effective<br>form for severe<br>stages of xerophthalmia<br>4. By-passes GI tract and<br>hence assurance is<br>obtained that it is not<br>lost by emesis. | 1. Most <u>expensive</u> form<br>2. Requires sterile<br>syringe<br>3. Requires some skill<br>to administer<br>4. Ampules must be<br>given handling care<br>as they are subject<br>to breakage<br>5. Not usually a field<br>form |





### Food Fortification Forms

Bulk vitamin A products must be put into some application form for the addition to foods. These forms have been previously described in this report (pages 13-23). Essentially, it involves a liquid or dry product composition which can be added to the food product during the batch or continuous process of manufacture. The vitamin product added to food, particularly when in dry form, is referred to as a premix. While this, at first sight, may seem to be a simple preparation, the ensuing discussion will point up the various ramifications which may have to be considered in its preparation and use. If a premixing is necessary, the manufacturer must decide whether to prepare the premix in his own plant or buy the premix from an "outside" premix manufacturer (72).

In choosing between making or buying his premix, a food manufacturer should consider the total costs of both approaches - not merely the price of the commercial premix vs. the costs of the premix ingredients. In evaluating a company's economic and manufacturing abilities to make its own premix, close cooperation between the technical, manufacturing and purchasing functions is necessary. Microingredients must be mixed with accuracy and carefully handled to avoid contamination or waste. Every function involved in the premixing operation can significantly affect the economics of the make vs. buy decision. The following costs should be considered in making an in-plant premix: (a) purchasing individual





bulk ingredients, (b) initial quality control of the single ingredients, (c) inventory of single ingredients, (d) labor and equipment costs in making in-house premix, (e) quality control costs to monitor production of in-house premix, (f) mechanical loss of material in mixer, conveyor, etc. (g) packaging costs of a premix and (h) supervisory personnel over the entire operation.

Inventory costs and losses depend largely on the experience and facilities available for handling and storing the single vitamins. Care must be taken to insure that employees don't treat costly bulk vitamins as perfunctorily as flour or salt.

Quality control costs first arise in the initial formulation of the premix and the establishment of the specific micronutrient forms to be used. Since various ingredients will be arriving from several manufacturers, a major quality control cost arises in the analysis of every ingredient before batching a premix. Such analysis demands skilled technicians with specialized lab equipment. For example, a vitamin A assay should be accurate to  $\pm 3\%$ . The instrumentation alone is costly.

In assembling the premix, accurate measuring and special mixing equipment must be used. Once the premix has been produced, the manufacturer must test it to insure that mixing and distribution objectives have been achieved. Manufacturing losses in the premixing operation frequently become the most difficult to control "leaks" in the premixing economics. Losses due to spillage, product hang up in mixing and conveying equipment, losses through dust, etc. vary greatly with equipment quality, equipment construction and operator expertise. Close planning is needed to determine the precise rate at



which premix should be manufactured to minimize inventory and warehousing costs, yet insure an ample on-hand supply to meet variations in production requirements. If the premix will be used in more than one plant location, allowances must be made for shipping the premix to meet production schedules.

A strong reason for buying a commercial premix from a reputable vitamin supplier is the assurance this provides of nutrient potency. Commercial premixes, such as those of Roche, are prepared to meet stringent standards. Commercially prepared premix reduces inventory control to simply another ingredient for the product being fortified. By purchasing a premix ready made to his specifications, the food manufacturer avoids an investment in premixing equipment, personnel and overhead. One of the best ways to evaluate the comparative costs in making an in-house premix or buying a commercially prepared premix is to analyze the total costs involved in the two approaches. The accompanying cost table (Table 27) can serve as a guide to this analysis (62).

Once one has settled on premix preparation or premix purchase, the only remaining stages for completing the food fortification process are (a) the acquisition of a suitable metering device to synchronize the introduction of the weight or volume of premix into the weight or volume of the food product to be followed by (b) an adequate blending or mixing operation to assure the final uniformity of the fortified product. Over the past thirty year or more, equipment has been manufactured to achieve this purpose. There are





liquid spraying devices for liquid products and dry ingredient micro-nutrient feeding devices which will feed into the food product, the appropriate addition of the vitamin premix. As an example, in the enrichment of white flour practiced for the past thirty years, as small a volume as 1/4 of an ounce (7 grams) of premix can be evenly introduced into 100 lbs. (45 kilograms) of flour. Usually, the decision as to what equipment is to be used in a food manufacturing operation has to be decided by the production engineers of the food company in consultation with food fortification experts and hence, the specific costs are somewhat influenced by these considerations and the existing food manufacturing process in the specific plant. In any case, however, the cost of the mechanical feeding or spraying equipment is a very small one compared to the equipment used in manufacturing the food product.

Because of years of experience in vitamin fortification of food and the concurrent development in the engineering of new mechanical equipment, there is no lack of technical or engineering knowledge today in adding vitamins to food products.

#### Goal and Approach to Xerophthalmia Eradication

Inquiry has been raised as to why xerophthalmia exists in the world today when it has been known for fifty five years that a deficiency of vitamin A causes xerophthalmia or blindness and adequate vitamin A supplies have been available for twenty five years or more. During the





last ten years it has been demonstrated that massive dosing of children twice a year, an emergency type practice, will prevent xerophthalmia. Fortification of food with vitamin A has been the best long range practice to prevent vitamin A deficiency in many parts of the world. The reason that xerophthalmia still exists is that the goal of xerophthalmia eradication has not received high priority and a coordinated plan for each country suffering from this vitamin deficiency disease has not been set in motion for the attainment of the goal at a specified time period.

One can, for the purpose of illustration, take a simple analogy which all mankind should understand. Man requires water. If he doesn't get water, he will become thirsty. This is a sensation of dryness in the mouth and throat associated with the need for water. More severe deprivation of this essential liquid will produce a dehydrated body with its attendant symptoms and finally, death. Now, today, if we were to find a population segment in any country suffering from the syndrome of water deficiency, would one first spend an extended time period conducting detailed surveys to ascertain the variation of the syndrome as it might be complicated by activity of the subject, seasonal influences, etc. Or would one, because of the need for water, send in and administer water after a quick confirmation of the need, the status of existing potable water facilities, and distribution pattern of water to the populace?

The same situation exists in man with vitamin A and vitamin A



deprivation. Man requires vitamin A. If he doesn't get vitamin A, he first develops night blindness, then the stages of xerophthalmia with its more non-reversible keratomalacia and finally, death. We must get away from the pitfall of having to reestablish in each population group man's basic need for vitamin A and its relationship to xerophthalmia, while thousands of youngsters continue to become blind because of the continuous plodding activity of those who wish to survey and study, and resurvey and ponder the routes of solutions to the problem.

If a dynamic approach is to be taken on this very specific matter of xerophthalmia eradication, it would seem that any country or population segment suspecting xerophthalmia as the cause of blindness in its children (where it usually first occurs) would proceed somewhat as follows:

1. Solicit physicians opinions or reports for incidence of the eye syndrome in the area involved or have a competent person check the eyes of an appropriate sampling of the population.
2. Make vitamin A determinations of the liver from children after death from the syndrome.
3. Run blood vitamin A determinations of living children with advanced symptoms (an appropriate sampling).
4. Check the dietary pattern of children with xerophthalmia tendency (an appropriate sampling).





5. With items 1-4 confirming vitamin A deficiency (at most, a 90 day study) completed, then plan for emergency treatment of children (and adults, if necessary) by the massive dosing technique in order to save the sight of as many as possible. Massive dosing should be a community monitored affair with records kept of participants and with personnel present to give out and confirm the ingestion of the preparation. This operation can usually be combined with a health check, a vaccination clinic, a malaria prevention treatment, etc.
6. For a more permanent solution to xerophthalmia, a more lengthy dietary study of the population must be made to see what foods naturally high in vitamin A can be introduced into the diet or what foods presently consumed by all can be fortified with vitamin A, as assurance in the future against the reoccurrence of the deficiency syndrome. With the establishment of an adequate daily intake of vitamin A, the emergency massive dosing can be discontinued. The dietary study of the population should preferably consider other nutrients such as protein, other vitamins and minerals. An inadequate protein and vitamin E intake can influence vitamin A utilization. Furthermore, with more complete knowledge on hand on other dietary inadequacies,





those in charge may wish to consider a multi-nutrient dietary supplement like "Nutricube" as a useful intermediate solution between the stages of (a) intermittent massive dosing of vitamin A and (b) the initiation of food fortification.

Those involved in xerophthalmia eradication might keep the objective of costs well in mind. The price of 10 cents (USA) or less buys a supply of vitamin A adequate to prevent blindness in a child for one year. With funds always limited for public health measures, let more be spent for getting this vital nutrient to the deficient child and less in monetary and time consuming activities involved in studying and surveying the overall problem beyond that necessary to confirm the need and the solution. Certainly for a few cents involved, the retention of the gift of sight should be a rewarding return of great magnitude to both the individual and the country concerned.

At the present time, there is no world-wide organization which has assumed the leadership of coordinating the programs in each country of the varied governmental and private efforts directed against the eradication of xerophthalmia. Such a coordinating role should be established for the more rapid achievement of the goal of xerophthalmia eradication.



### SUMMARY

Xerophthalmia, a result of vitamin A deficiency, now recognized for over fifty years, is still an important human affliction world-wide, particularly in children between one and six years of age. Vitamin A is not synthesized in the body and must be supplied exogenously. The occurrence precursors and metabolism of vitamin A, a micronutrient required in the diet for adequate nutrition in man and animals, are well understood and data exist on its functions, deficiency symptoms and requirements. Vitamin A can be either (a) consumed daily to meet physiological needs or (b) consumed intermittently in amounts beyond daily needs, most of the excess vitamins being absorbed, transported and stored in the liver for future tissue needs.

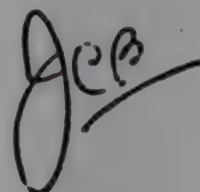
1972 is the 25th anniversary year of the production of vitamin A by chemical synthesis in virtually unlimited quantities. It is available as retinol, (vitamin A alcohol) but is usually incorporated in food as the retinyl esters in the form of the palmitate or the acetate. Descriptions as well as physical and chemical data exist, on these products. Since vitamin A is subject to oxidation accelerated by heat, light and certain catalysts, a technology of application forms has been developed by which the oxidative tendency is inhibited or delayed by physical and chemical means. Thus an array of application-market forms are available with their respective specifications and uses.

In an emergency or short term approach, a single massive oral or parenteral dose of vitamin A may be administered to the child twice a year for the prevention of vitamin A deficiency. For the normal or long-term approach vitamin A should be provided in the daily diet by the fortification of regularly consumed, indigenous foods of the concerned population. Past experience indicates that vitamin A may be added to a variety of liquid and dry foods without flavor problems, with adequate stability and full biological availability of the incorporated vitamin at low cost.





Even though large volume production of vitamin A has existed at favorable cost figures for some years, significant populations of the world suffer because of its lack. The problems of resistance to vitamin supplementation as public health measures, unsuccessful or inadequate education programs, and particularly distribution systems, need to be overcome to wipe out blindness due to vitamin A deficiency. The goal of xerophthalmia eradication must have high priority and a dedicated plan of approach with a time schedule for its completion.

A handwritten signature in dark ink, consisting of the letters 'JCB' followed by a long, sweeping horizontal stroke that extends to the right.

JCB:cs

Hoffmann-LaRoche, Inc.  
January 2, 1973  
Revised





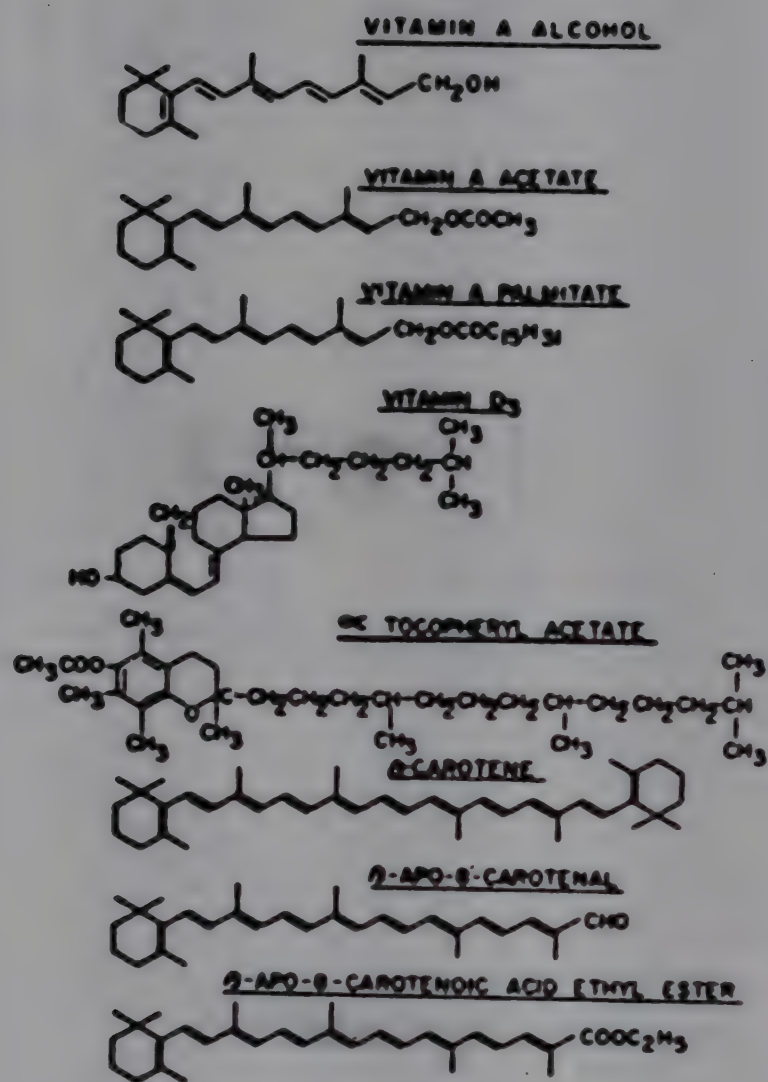


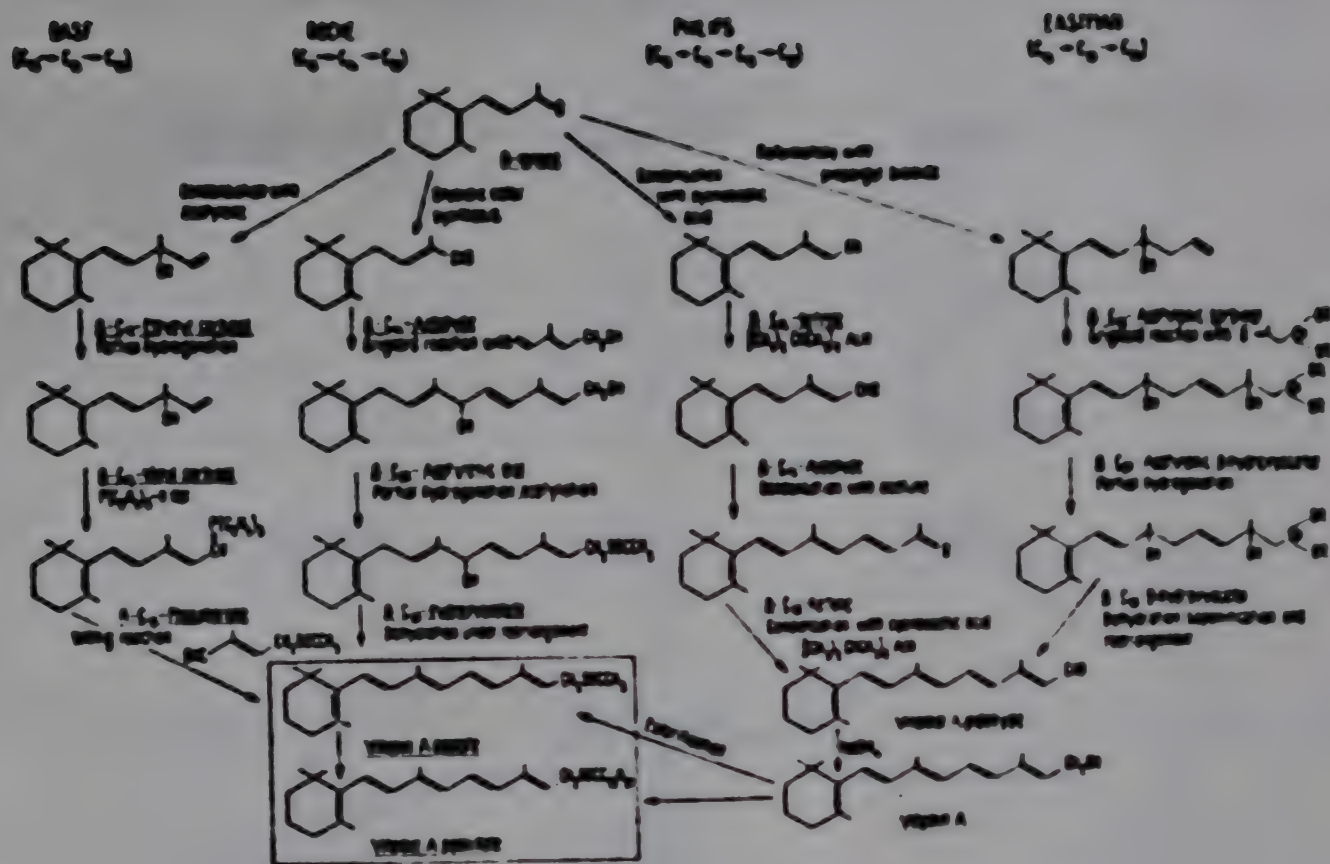
Figure 1. Structural formulae of vitamin A, some carotenoid vitamin A precursors and other fat-soluble vitamins.











**Figure III. Technical syntheses of vitamin A esters. (6)**





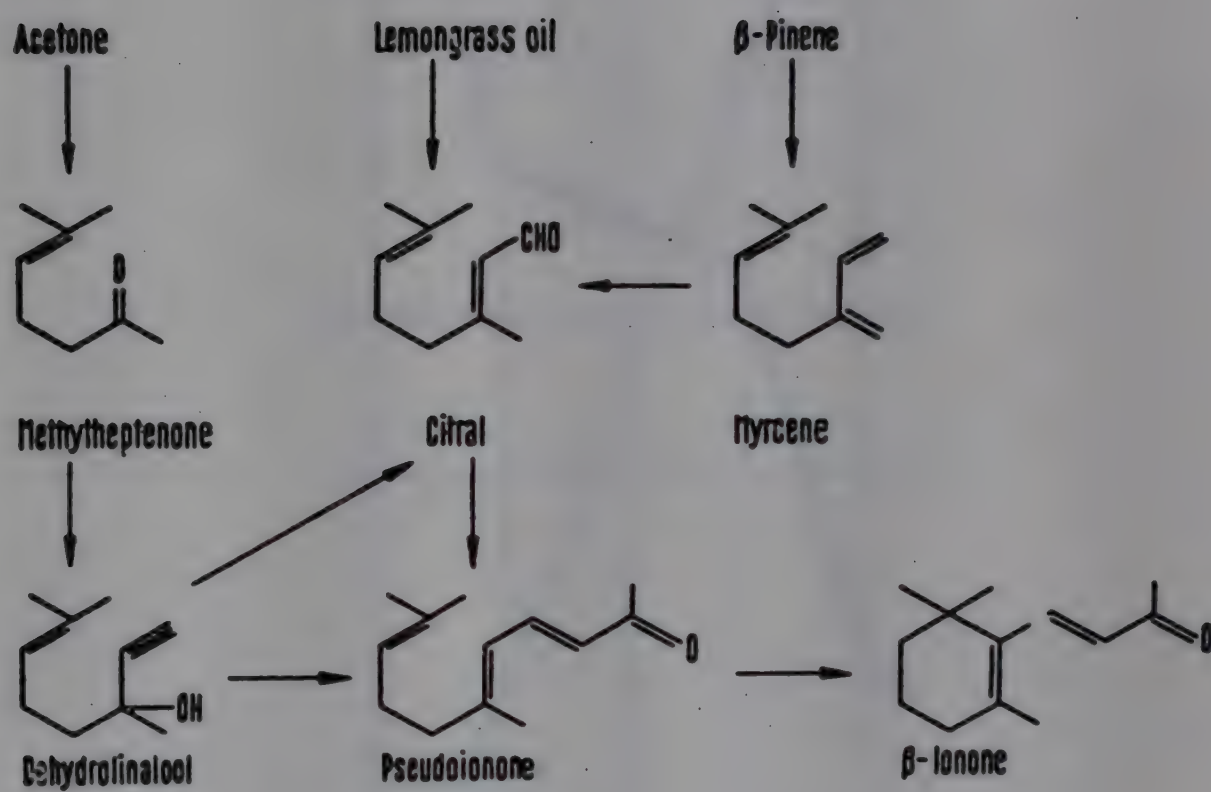


Figure IV. Technical synthesis of  $\beta$ -ionone. (6)



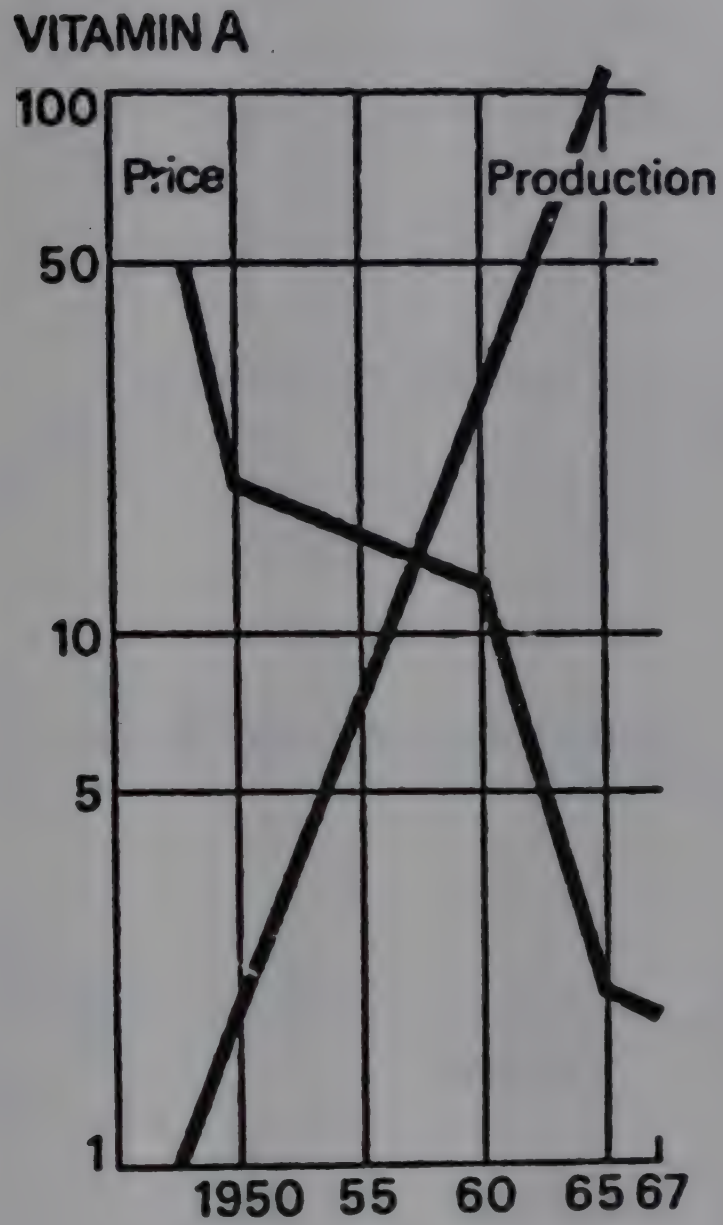


Figure V. Logarithmic production - price pattern of vitamin A with time.





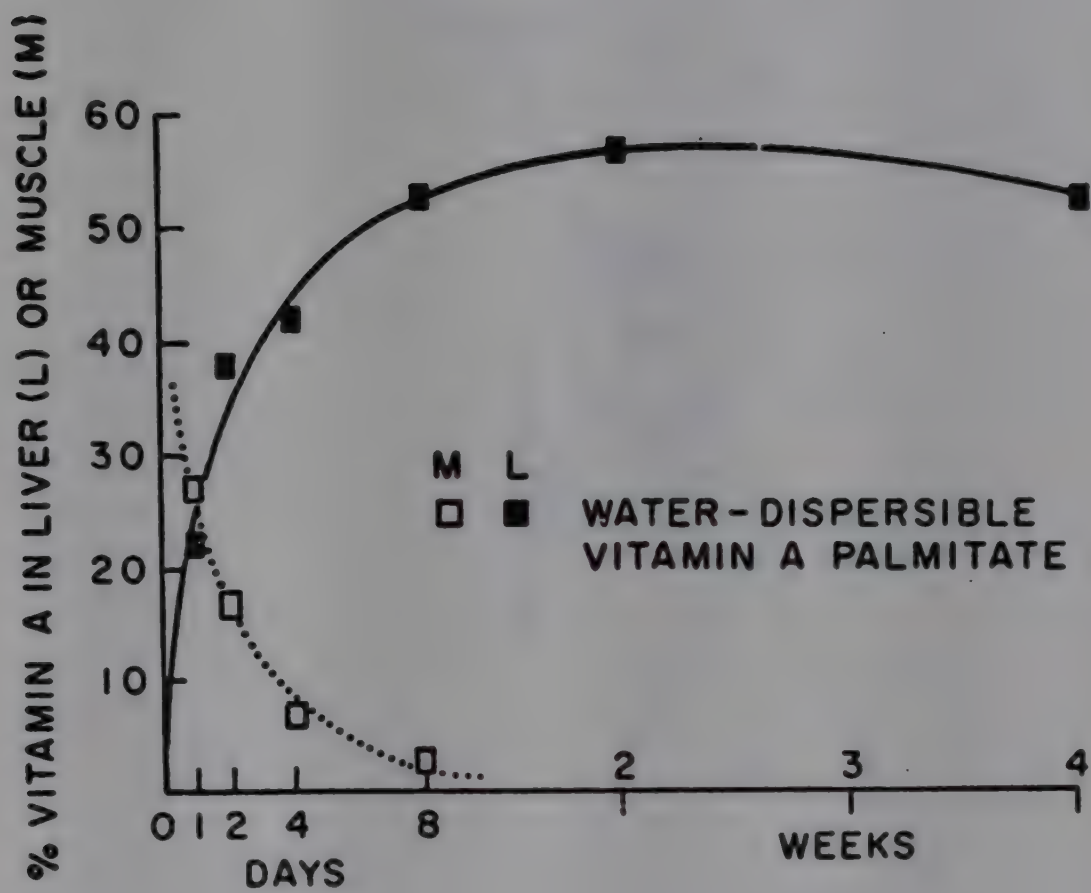


Figure VI. Translocation pattern of an effective water-dispersible vitamin A formulation injected IM in the muscle to the liver in the chicken.





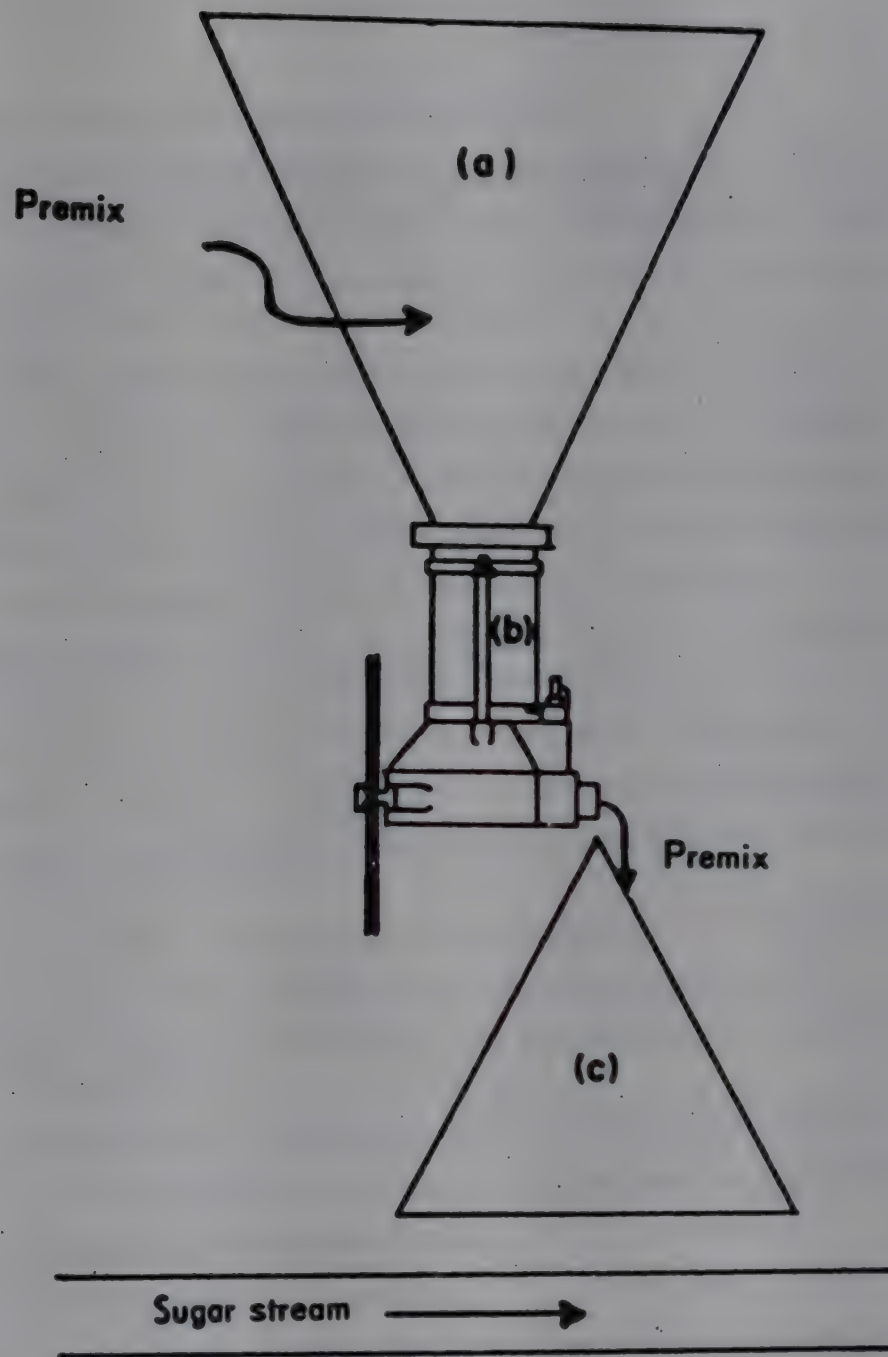


Figure VII. Sugar Fortification Equipment (68)



96  
Table 1

Some Historical Events on Vitamin A and Provitamins A

|         |                                                                                                                                      |
|---------|--------------------------------------------------------------------------------------------------------------------------------------|
| 1831    | Carotene isolated from carrots (Wackenroder)                                                                                         |
| 1906-11 | Column chromatography developed for carotenoid separation (Tswett)                                                                   |
| 1907    | Empirical formulae of carotene established (Willstätter and Mieg)                                                                    |
| 1913-15 | Fat-soluble growth factor "A" recognized in cod liver oil and butter (McCollum and Davis, Osborne and Mendel)                        |
| 1917    | Xerophthalmia recognized as vitamin A deficiency (McCollum and Simmonds)                                                             |
| 1919    | Growth factor observed in liver for poultry (Palmer and Kempster)                                                                    |
| 1919-20 | Vitamin A activity related to yellow carotenoids of corn (Steenbock and coworkers)                                                   |
| 1921    | Quantitative rat growth assay method developed for vitamin A (Zilva and coworkers)                                                   |
| 1923    | Poultry shown to require vitamin A (Emmett and Peacock)                                                                              |
| 1920-25 | Vitamin activity in fish oil associated with purple color reaction of sulphuric acid or arsenic trichloride (Rosenheim and Drummond) |
| 1924-26 | Ruminants shown to require vitamin A (Hart et al. and Jones et al.)                                                                  |
| 1926    | Stable color with antimony trichloride in chloroform (Carr and Price)                                                                |
| 1928    | Carotene gives Carr-Price reaction and cures A deficiency in rats (von Euler and coworkers)                                          |
| 1929-31 | Structure of carotene and vitamin A established (Karrer and coworkers)                                                               |
| 1930    | Carotene demonstrated to be converted to vitamin A (Moore)                                                                           |
| 1931    | First vitamin A standard developed: $0.6 \mu\text{g } \beta\text{-carotene} = 1 \text{ IU vitamin A}$ (League of Nations)            |
| 1937-40 | Vitamin A crystallized (Holmes and Corbett, Baxter and Robeson)                                                                      |
| 1947    | Vitamin A synthesized (Isler and coworkers, Arens and Van Dorp)                                                                      |
| 1948    | Vitamin A <sub>2</sub> isolated (Salah and Morton)                                                                                   |
| 1949-50 | Commercial production of vitamin A                                                                                                   |
| 1951-52 | Development of gelatin beadlet stabilized vitamin A for animal applications                                                          |
| 1949-53 | Modification of vitamin A in vision revealed (Wald and Ball and Morton)                                                              |
| 1950    | Present vitamin A standard adopted: $0.0344 \mu\text{g vitamin A acetate} = 1 \text{ IU}$ (World Health Organization)                |
| 1950    | Synthesis of $\beta\text{-carotene}$ (Karrer and coworkers, Inhoffen and coworkers, Milas and coworkers)                             |
| 1951    | Vitamin A <sub>2</sub> synthesized (Farrar and coworkers)                                                                            |
| 1954-56 | Industrial synthesis of $\beta\text{-carotene}$ (Isler and coworkers); Commercial production of $\beta\text{-carotene}$              |





- 1958-59      Synthesis of  $\beta$ -apo-8'-carotenal (Isler et al. and Rüegg et al.)
- 1970          Several quadrillion ( $10^{15}$ ) International Units of vitamin A are produced by chemical synthesis annually

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Table 2

## Vitamin A in Various Fish Liver Oils (4)

| <u>Source of oil</u> | <u>Zoological name</u>      | <u>Potency</u><br>(IU/g) |
|----------------------|-----------------------------|--------------------------|
| Haddock              | Gadus algelefinus           | 65                       |
| Cod                  | Gadus morrhua               | 600                      |
| Striped bass         | Roccus lineatus             | 4,500                    |
| Jack smelt           | Atherinopsis californiensis | 10,000                   |
| Albacore             | Germo alalunga              | 18,000                   |
| Grouper              | Epinephelus morio           | 25,000                   |
| Boston mackerel      | Scomber scombrus            | 30,000                   |
| Barracuda            | Sphyræna argentea           | 40,000                   |
| Skipjack tuna        | Katsuwonus pelamis          | 40,000                   |
| Yellowtail           | Seriola dorsalis            | 50,000                   |
| White sea-bass       | Cynoscion nobilis           | 50,000                   |
| Red snapper          | Lutianus campechanus        | 60,000                   |
| Totuava              | Eriscion macdonaldi         | 60,000                   |
| Bluefin tuna         | Thunnus thynnus             | 60,000                   |
| Yellowfin tuna       | Neothunnus macropterus      | 70,000                   |
| Pacific mackerel     | Pneumatophorus diego        | 80,000                   |
| Bonito               | Sarda chiliensis            | 120,000                  |
| Oxbrilla pinta       | Epinephelus analogus        | 170,000                  |
| Swordfish            | Xiphias gladius             | 250,000                  |
| Ishnagi              | Stereolepis ishnagi         | 300,000                  |
| Black sea-bass       | Stereolepis gigas           | 600,000                  |



Table 3  
Carotene Content of Foods\*

| Food                     | Carotene (mg/kg) |     |      | Vitamin A equivalent (IU/g) |     |     | Food                      | Carotene (mg/kg) |      |        | Vitamin A equivalent (IU/g) |      |        |
|--------------------------|------------------|-----|------|-----------------------------|-----|-----|---------------------------|------------------|------|--------|-----------------------------|------|--------|
|                          | (A)              | (B) | (C)  | (A)                         | (B) | (C) |                           | (A)              | (B)  | (C)    | (A)                         | (B)  | (C)    |
| Apples (raw)             | 0.3              | 0.5 | 0.9  |                             |     |     | Beans, runner (boiled)    | 3                | -    | -      |                             | -    | -      |
| Apricots (raw)           | 15               | 25  | 27   |                             |     |     | Beetgreens (boiled)       | 50               | 83   | 51     |                             | 83   | 51     |
| Apricot nectar (canned)  |                  |     |      |                             |     |     | Broccoli (boiled)         | 25               | 42   | 25     |                             | 42   | 25     |
| Apricots, dried (raw)    | 36               | 60  | 109  |                             |     |     | Brussel sprouts (boiled)  | 4                | 6.7  | 5.2    |                             | 6.7  | 5.2    |
| Apricots, dried (stewed) | 12               | 20  |      |                             |     |     | Cabbage                   | 3                | 5    | 1.3    |                             | 5    | 1.3    |
| Avocados (raw)           | 1                | 1.7 | 2.9  |                             |     |     | Carrots (canned)          | 70               | 116  | 105    |                             | 116  | 105    |
| Bananas (raw)            | 2                | 3.3 | 1.9  |                             |     |     | Cauliflower (boiled)      | 0.3              | 0.5  | 0.6    |                             | 0.5  | 0.6    |
| Blackberries (raw)       | 1                | 1.7 | 2    |                             |     |     | Celery (raw)              | 20               | 33   | 33     |                             | 33   | 33     |
| Cherries (raw)           | 1.2              | 2   | 1.1  |                             |     |     | Chicory greens (raw)      | -                | -    | 40     |                             | -    | 40     |
| Cherries, sour (canned)  | 5.0              | 8.3 | 6.6  |                             |     |     | Chives (raw)              | -                | -    | 58     |                             | -    | 58     |
| Currents, black (raw)    | 2                | 3.3 | 2.3  |                             |     |     | Collards (boiled)         | -                | -    | 78     |                             | -    | 78     |
| Gooseberries (raw)       | 1.8              | 3   | 2.9  |                             |     |     | Corn, yellow (boiled)     | -                | -    | 4      |                             | -    | 4      |
| Melons, yellow (raw)     | 20               | 33  | 34   |                             |     |     | Corn meal, yellow         | -                | -    | 5      |                             | -    | 5      |
| Mangos, (raw)            | -                | -   | 48   |                             |     |     | Dandelion greens (boiled) | -                | -    | 117    |                             | -    | 99     |
| Nectarines (raw)         | -                | -   | 16.5 |                             |     |     | Endive (raw)              | 20               | 33   | 33     |                             | 33   | 33     |
| Olives, green (canned)   | 1.5              | 2.5 | 3    |                             |     |     | Kale (boiled)             | 50               | 83   | 83     |                             | 83   | 83     |
| Oranges and Juice (raw)  | 0.5              | 0.8 | 2    |                             |     |     | Lettuce (raw)             | 10               | 17   | 3.3-19 |                             | 17   | 3.3-19 |
| Peaches (raw)            | 5                | 33  | 13.3 |                             |     |     | Mint (raw)                | 110              | 183  | -      |                             | 183  | -      |
| Peaches, dried (raw)     | 20               | 33  | 39   |                             |     |     | Mustard greens (raw)      | 50               | 83   | 58     |                             | 83   | 58     |
| Pears (raw)              | 0.1              | 0.2 | 0.2  |                             |     |     | Okra (boiled)             | -                | -    | 4.9    |                             | -    | 4.9    |
| Pineapples               | 0.6              | 1   | 0.7  |                             |     |     | Parsley (raw)             | 80               | 133  | 85     |                             | 133  | 85     |
| Plums (raw)              | 2.2              | 3.7 | 3    |                             |     |     | Peas (boiled)             | 3                | 5    | 5.4    |                             | 5    | 5.4    |
| Prunes, dried (raw)      | 10               | 17  | 21.7 |                             |     |     | Peas, dried (boiled)      | 0.8              | 1.3  | 0.4    |                             | 1.3  | 0.4    |
| Prunes, dried (stewed)   | 5                | 8.3 | 7.6  |                             |     |     | Spinach (boiled)          | 60               | 100  | 81     |                             | 100  | 81     |
| Raspberries (raw)        | 0.8              | 1.3 | 0.3  |                             |     |     | Squash, summer (boiled)   | -                | -    | 3.9    |                             | -    | 3.9    |
| Rhubarb (raw)            | 0.6              | 1   | 0.8  |                             |     |     | Squash, winter (boiled)   | -                | -    | 35     |                             | -    | 35     |
| Strawberries (raw)       | 0.3              | 0.5 | 0.6  |                             |     |     | Sweet potatoes (boiled)   | -                | -    | 79     |                             | -    | 79     |
| Tangerines (raw)         | 1                | 1.7 | 4.2  |                             |     |     | Tomatoes or juice (raw)   | 7                | 11.7 | 8-10   |                             | 11.7 | 8-10   |
| Asparagus (boiled)       | 5                | 8.3 | 9    |                             |     |     | Tomatoes (canned)         | 5                | 8.3  | 9      |                             | 8.3  | 9      |
| Beans, french (boiled)   | 5                | 8.3 | 5.4  |                             |     |     | Turnip greens (boiled)    | 60               | 100  | 63     |                             | 100  | 63     |
|                          |                  |     |      |                             |     |     | Water cress (raw)         | 30               | 50   | 49     |                             | 50   | 49     |

\*(A) Carotene content from McCance, R. A. and Widdowson, E. M. The Composition of Foods, Med. Res. Council Spec. Rept. Ser. 297, England (1960)

(B) Vitamin A values calculated from above reference on basis of 1 mcg = 1667 IU.

(C) Vitamin A values from Watt, B. K. and Merrill, A. L. Composition of Foods, USDA-ARS Agr. Handbook #8(1963)





Table 4

Ratios of Biological Effectiveness of  $\beta$ -Carotene and Vitamin A  
for Various Animals (5)

| <u>Animal</u> | <u>Authority<sup>1</sup></u> | <u>mg <math>\beta</math>-carotene<sup>2</sup><br/>equivalent to 1 mg<br/>vitamin A alcohol</u> | <u>IU<sup>3</sup> preformed<br/>vitamin A per<br/>mg <math>\beta</math>-carotene</u> |
|---------------|------------------------------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Rat           | International                | 2                                                                                              | 1667                                                                                 |
| Mink          | NAS-NRC                      | 12                                                                                             | 277                                                                                  |
| Fox           | NAS-NRC                      | 12                                                                                             | 277                                                                                  |
| Dog           | NAS-NRC                      | 4 or 8                                                                                         | 418 - 834                                                                            |
| Poultry       | NAS-NRC                      | 2                                                                                              | 1667                                                                                 |
|               | NCAN                         | 3                                                                                              | 1112                                                                                 |
|               | ARC                          | 6                                                                                              | 556                                                                                  |
| Horse         | NAS-NRC                      | 6 - 10                                                                                         | 333 - 556                                                                            |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |
| Dairy Cattle  | NAS-NRC                      | 8 - 10                                                                                         | 333 - 418                                                                            |
|               | ANRC                         | 8                                                                                              | 418                                                                                  |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |
| Beef Cattle   | NAS-NRC                      | 8.3                                                                                            | 400                                                                                  |
|               | ANRC                         | 8                                                                                              | 418                                                                                  |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |
| Sheep         | NAS-NRC                      | 5.8 - 8.3                                                                                      | 400 - 578                                                                            |
|               | ANRC                         | 8                                                                                              | 418                                                                                  |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |
| Swine         | NAS-NRC                      | 6.2                                                                                            | 533                                                                                  |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |
| Man           | MRC                          | 6                                                                                              | 556                                                                                  |
|               | NAS-NRC                      | 4                                                                                              | 834                                                                                  |
|               | BMA                          | 6                                                                                              | 556                                                                                  |
|               | CCN                          | 8                                                                                              | 417                                                                                  |
|               | NCAN                         | 7                                                                                              | 476                                                                                  |

<sup>1</sup> ANRC, Animal Nutrition Research Council, N. America; ARC, Agricultural Research Council of Great Britain; BMA, British Medical Association; CCN, Canadian Council on Nutrition; MRC, Medical Research Council, Great Britain; NAS-NRC, National Academy of Sciences, National Research Council, United States; NCAN, National Committee on Animal Nutrition, Canada.

<sup>2</sup> On the basis of all-trans  $\beta$ -carotene. <sup>3</sup> 1 IU of vitamin A activity is usually regarded as  $\approx$  to 0.6  $\mu$ g  $\beta$ -carotene or 0.3  $\mu$ g vitamin A alcohol or 0.344  $\mu$ g of vitamin A acetate or 0.55  $\mu$ g of vitamin A palmitate.





Table 5

## Functions of Vitamin A

1. Maintenance of proper vision.
2. Maintenance of spermatogenesis in the male.
3. Maintenance of the placenta and prevention of resorption of the fetus in the female.
4. Maintenance of bone development and growth.
5. Maintenance of the mucus-secreting cells of epithelia, the biosynthesis of glycoproteins and the prevention of keratinization.
6. Interaction with vitamin E in regulating stability of biological membranes.
7. Involved in production of corticosteroids and in glycogenesis.
8. Interrelated with thyroid hormone function.
9. Influences synthesis of serum and muscle proteins.



Table 6

## Deficiency Symptoms of Vitamin A

## General

Cessation of growth  
 Cystic pituitary glands  
 Death  
 Decline in body weight  
 Diarrhea (scours)  
 Failure of appetite  
 General edema (anasarca)  
 Reduced resistance to parasites  
   Infections  
 Untidy hair or feathers  
 Xerosis of membranes

## Bone formation

Cancellous bone  
 Defective modeling  
 Narrowing of foramina  
 Restriction of brain cavity

## Congenital abnormalities

Anophthalmia  
 Aortic arch deformities  
 Cleft palate  
 Hydrocephalus  
 Kidney deformities  
 Microphthalmia

## Defective reproduction

Abnormal estrous cycle  
 Dead, weak or blind offspring  
 Degeneration of testes  
 Reduced egg production & hatchability  
 Resorption of fetuses

## Eyes

Keratomalacia  
 Lacrimation  
 Night blindness (nyctalopia)  
 Pyogenic infections  
 Xerophthalmia

## Eyes (continued)

Constriction of optic nerve  
 Loss of lens  
 Opacity of cornea  
 Papilloedema

## Intestinal tract

Enteritis  
 Metaplasia of fore stomach

## Liver

Degeneration of Kupffer cells  
 Metaplasia of bile ducts

## Nervous system

Constriction at foramina  
 Convulsive seizure  
 Hydrocephalus  
 Incoordination & staggering gait  
 Nerve degeneration  
 Paresis  
 Raised cerebrospinal fluid pressure  
 Twisting of nerve

## Respiratory system

Lung abscesses, caseation  
 Metaplasia of nasal passages  
 Nasal discharge  
 Pneumonia

## Urinary system

Cystitis  
 Nephrosis  
 Pus in ureters  
 Pyelitis  
 Thickening of bladder wall  
 Urolithiasis (urates)





Table 7

Requirements for Vitamin A and E for Infants, Children and Adults

| <u>Groups</u> | <u>Vitamin A</u>       |                       |                                                             | <u>Vitamin E</u>                                                    |                                                               |
|---------------|------------------------|-----------------------|-------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------|
|               | <u>Age</u><br>(months) | <u>Weight</u><br>(kg) | <u>NAS/NRC<sup>1</sup></u><br><u>RDA Values</u><br>(IU/day) | <u>FAO/WHO<sup>2</sup></u><br><u>Recommended Intake</u><br>(ug/day) | <u>NAS/NRC<sup>1</sup></u><br><u>RDA - Values</u><br>(IU/day) |
| Infants       | 0-2                    | 4                     | kgx120 or .480                                              | -                                                                   | 5                                                             |
|               | 2-6                    | 7                     | kgx110 or 770                                               | -                                                                   | 5                                                             |
|               | 6-12                   | 9                     | kgx100 or 900                                               | 300                                                                 | 5                                                             |
| Children      | (Years)                |                       |                                                             |                                                                     |                                                               |
|               | 1-2                    | 12                    | 1100                                                        | 250                                                                 | 10                                                            |
|               | 2-3                    | 14                    | 1250                                                        | 250                                                                 | 10                                                            |
|               | 3-4                    | 16                    | 1400                                                        | 250                                                                 | 10                                                            |
|               | 4-6                    | 19                    | 1600                                                        | 300                                                                 | 10                                                            |
| Young Male    | 18-22                  | 67                    | 5000                                                        | 750                                                                 | 30                                                            |
| Young Female  | 18-22                  | 58                    | 5000                                                        | 750                                                                 | 25                                                            |
| Pregnancy     | -                      | -                     | 6000                                                        | 750                                                                 | 30                                                            |
| Lactation     | -                      | -                     | 8000                                                        | 1250                                                                | 30                                                            |

<sup>1</sup>Recommended Dietary Allowances (selected age groups).  
NAS-NRC, Food & Nutrition Board Publication 1694 (1968).

<sup>2</sup>Requirements of Vitamin A, Thiamine, Riboflavin and Nicotin.  
FAO/WHO Expert Group Report FAO Report No. 41 or WHO Report No. 362.  
September (1967).



U.S. PRODUCTION & SALES - VITAMIN A  
(U.S. Tariff Commission)

|                                   | Quantity             |                 | Sales<br>Value<br>(\$000's) | Unit<br>Value<br>\$/BU |
|-----------------------------------|----------------------|-----------------|-----------------------------|------------------------|
|                                   | Production<br>(T.U.) | Sales<br>(T.U.) |                             |                        |
| Total Alcohol & Esters            |                      |                 |                             |                        |
| 1967                              | 970                  | 622             | \$ 16,720                   | \$26.88                |
| 1968                              | 1,064                | 740             | 18,593                      | 25.12                  |
| 1969                              | 1,192                | 899             | 22,584                      | 25.13                  |
| 1970                              | 1,274                | 853             | 21,222                      | 24.89                  |
| Vitamin A Palmitate<br>Feed Grade |                      |                 |                             |                        |
| 1967                              | 550                  | 434             | \$ 10,410                   | \$23.98                |
| 1968                              | 719                  | 472             | 10,239                      | 21.69                  |
| 1969                              | 843                  | 593             | 12,280                      | 20.70                  |
| 1970                              | 816                  | 563             | 11,660                      | 20.69                  |
| All Other Forms                   |                      |                 |                             |                        |
| 1967                              | 420                  | 188             | \$ 6,130                    | \$33.58                |
| 1968                              | 344                  | 268             | 8,354                       | 31.16                  |
| 1969                              | 349                  | 306             | 10,304                      | 33.72                  |
| 1970                              | 458                  | 289             | 9,562                       | 33.08                  |





Table 9

**Factors Influencing Vitamin A Stability**

**SENSITIVE TO:** Air  
Oxidizing Agents  
Acid (Destruction and Isomerization)  
Heat  
Moisture  
Trace Metals

**STABILIZED BY:** Antioxidants  
Protective Coatings  
Inert Gas or Vacuum Packing



TABLE 10

Stability of Vitamin A Concentrates

| <u>Concentrate</u>                      | <u>% retention of vitamin A</u>  |                                   |
|-----------------------------------------|----------------------------------|-----------------------------------|
|                                         | At 45°C, open, thin layer        | Room temperature<br>closed bottle |
|                                         | 8 weeks                          | 6 months                          |
| Dry Vitamin A Acetate<br>Type 500       | 87                               | 90                                |
| Dry Vitamin A Palmitate<br>Type 250 CWS | 87                               | 90                                |
| Type 250-SD                             | 77                               | 88                                |
| Type 250-S                              | 93                               | 94                                |
| Type 500                                | 85                               | 93                                |
|                                         | <u>24 hr/100°C Closed Bottle</u> |                                   |
| PIMO                                    | 90                               | 98-99                             |
| AIMO                                    | 85                               | 98-99                             |





Table 11

Vitamin A Determination in Liver, a Carr-Price Procedure

1. Each single or group of liver samples (total weight not over 20 grams) is put into a small metal Waring Blendor and 80 ml of aldehyde-free 6% alcohol KOH added. If liver weight is greater than 20 g, increase solvents proportionately.
2. The livers are blended for 5 minutes.
3. Immediately after the Blendor is stopped, the solution is poured into a 100 ml glass-stopped cylinder, cooled and the volume made to 100 ml with alcohol KOH. Mix thoroughly.
4. A 5 ml aliquot is pipetted into a glass-stoppered centrifuge tube and placed in a 75°C water bath for 30 minutes.
5. The tubes are cooled and 5 ml of distilled H<sub>2</sub>O and 20 ml of petroleum ether are added.
6. The tubes are given one shake by hand after the petroleum ether has been added.
7. The pressure is released and the tubes are then shaken for 3 minutes in a shaking machine. After shaking, the tubes are centrifuged for 1 minute.
8. A 3 ml aliquot of the petroleum ether is transferred to a clean Evelyn tube and evaporated with a mild stream of nitrogen. After all the petroleum ether has evaporated, 2 ml chloroform are added plus 2 droplets of acetic anhydride and the usual Carr-Price reaction with antimony trichloride solution in CHCl<sub>3</sub> is carried out.
9. If liver potencies are much different from the example given below the following may be employed. For livers of lower potency, the amount of petroleum ether used for extraction can be decreased to 10 ml and the aliquot taken for evaporation and Carr-Price reading can be increased to 5 ml. For livers of higher potency, the volume of the blended liver extract may be increased from 100 ml to a suitably higher volume, or the petroleum ether may be subdiluted before evaporation for Carr-Price reading.

Calculations

1. Convert galvanometer reading to L value from chart  
K = calibration constant

$$L \times K \times \frac{100}{5} \times \frac{20}{3} \times \frac{1}{4} = \text{units per liver}$$

2. Example

G reading = 62° · L = 0.2076; K = 52 with present equipment

$$0.2076 \times 52 \times \frac{100}{5} \times \frac{20}{3} \times \frac{1}{4} = 360 \text{ units per liver}$$



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Table 12

Vitamin A Determination in Blood, a Carr-Price Procedure

1. Since the carotenoids in blood also give a blue reaction with antimony trichloride, it was necessary to determine the carotene content of the blood. The vitamin A and carotene in the blood were extracted with purified petroleum benzine U.S.P. (petroleum ether), as recommended by Clausen and McCoord.
2. To 3 to 5 cc. of blood plasma 5 cc of 95 per cent ethyl alcohol and 8 cc. of purified petroleum benzine were added. The mixture was shaken for ten minutes and then centrifuged. Six cubic centimeters of the supernatant purified petroleum benzine extract was measured for its carotene content as 440 micromicrons with an Evelyn photoelectric colorimeter. The number of micrograms of carotene was determined by means of a calibration curve with a sample of pure carotene supplied to us by the General Biochemicals Company, Cleveland.
3. The purified petroleum benzine was evaporated in a water bath at 70 C., a stream of nitrogen being passed through the liquid during evaporation. The oily residue was dissolved in 0.6 cc. of chloroform, and 5.4 cc. of a 25 per cent solution of antimony trichloride (in chloroform) was added. The intensity of the resulting blue color was measured at 620 micromicrons in the Evelyn photoelectric colorimeter. The reading was translated into biologic IU or USP units, by referring to a calibration curve obtained with a sample of pure vitamin A of known biologic potency.







The vitamin A content of the plasma was calculated after subtracting the value for carotene.

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Comments on Liver and Blood Vitamin A

The liver acts as a storage depot for vitamin A. It rapidly takes up vitamin A absorbed through the intestinal walls when excesses over current body needs are provided in the ration. Conversely, it releases vitamin A to the blood stream when dietary intake falls below current needs. The amount of vitamin A at any given time in the liver, known as the hepatic reserve, will vary according to the amount of previous effective vitamin A intake. An adequate reserve of vitamin A is required for protection against periods of inadequate intake or impaired absorption—as well as against increased demands caused by stress and disease.

The vitamin A level in the blood is normally quite uniform for a given species and individual, regardless of the dietary intake and of the amount of liver reserves (unless liver reserves approach exhaustion). Only when the liver reserve becomes dangerously low and the individual is receiving inadequate vitamin A does the blood reflect the liver status. Hence, blood values alone are not a reliable practical index of the vitamin A reserves of an individual. Subjective observations of the individual and their dietary history or survey are also important.



Table 13

Retention of Added Vitamin A in Margarine  
(IU of Vitamin A per pound by Carr-Price assay)

| Manufacturer | Source of Vitamin A                                                       | Initial Assay | After six weeks storage |              | After twelve weeks storage |              | After 4 mths. storage |                             | After 6 mths storage      |                          |
|--------------|---------------------------------------------------------------------------|---------------|-------------------------|--------------|----------------------------|--------------|-----------------------|-----------------------------|---------------------------|--------------------------|
|              |                                                                           |               | 72° F.                  | 86° F.       | 40° F.                     | 40-72° F.    | 72° F.                | 86° F.                      | 40° F.                    | 40° F. 72° F.            |
|              |                                                                           |               |                         |              |                            |              |                       |                             |                           |                          |
| A            | Fish oil concentrate                                                      | 20,300        | ...                     | ...          | ...                        | 18,600 (92)  | 18,200 (90)           | 18,200 (90)                 | 18,500 (91)               | 17,500 (86)              |
| A            | Synthetic vitamin A acetate <sup>3</sup> 200,000 U.S.P. units per gram    | 21,250        | ...                     | ...          | ...                        | 20,200 (95)  | 19,500 (92)           | 20,900 (98)                 | 18,800 (88)               | 19,000 (89)              |
| A            | Synthetic vitamin A palmitate, type PIMO, 1,000,000 U.S.P. units per gram | 17,000        | 16,900 (99)             | 17,200 (101) | ...                        | 18,700 (110) | 18,000 (106)          | ...                         | 15,500                    | ....                     |
| B            | Fish oil concentrate                                                      | 19,350        | 17,450 (90)             | 17,650 (91)  | 17,100 (88)                | 18,000 (93)  | 17,200 (89)           | 15,600 (81)                 | 16,600 <sup>5</sup> (86)  | 16,900 <sup>2</sup> (87) |
| B            | Synthetic vitamin A acetate <sup>3</sup> 200,000 U.S.P. units per gram    | 14,550        | 14,150                  | 14,000       | 14,900                     | 14,900       | 15,000                | 14,500                      | 15,300 <sup>5</sup>       | 14,900 <sup>2</sup>      |
| C            | Synthetic vitamin A acetate <sup>3</sup> 200,000 U.S.P. units per gram    | 14,500        | 14,500 (100)            | 14,000 (97)  | 14,600 (101)               | 14,900 (103) | 14,700 (101)          | 15,500 (107)                | 16,100 (111)              | ...                      |
| D            | Synthetic vitamin A acetate <sup>3</sup> 100,000 U.S.P. units per gram    | 19,500        | 18,900 (97)             | 17,200 (88)  | ...                        | 20,200 (104) | 19,500 (100)          | ...                         | 19,300 <sup>4</sup> (106) | 22,400 (115)             |
| D            | Synthetic vitamin A acetate <sup>3</sup> 400,000 U.S.P. units per gram    | 17,600        | 18,200 (103)            | ...          | ...                        | 18,900 (107) | 19,300 (110)          | ...                         | 18,600 (106)              | 18,800 (107)             |
| E            | Fish oil concentrate                                                      | 16,900        | 18,400 (109)            | 18,300 (108) | ...                        | 17,600 (104) | ...                   | 18,500 (110)                | ...                       | 18,100 (107)             |
| E            | Synthetic vitamin A acetate, type AIMO, 1,000,000 U.S.P. units per gram   | 16,200        | 16,300 (101)            | 16,100 (99)  | ...                        | 17,500 (108) | ...                   | 17,600 <sup>4,6</sup> (109) | 17,200 (106)              | ...                      |





Table 13 (continued)  
Retention of Added Vitamin A in Margarine  
(IU of Vitamin A per pound by Carr-Price assay)

| Manufacturer | Source of Vitamin A Assay                            | Initial |  | After six weeks storage |  | After twelve weeks storage |  | Aft. 4 mths. storage |  | After 6 mths. storage     |  |
|--------------|------------------------------------------------------|---------|--|-------------------------|--|----------------------------|--|----------------------|--|---------------------------|--|
|              |                                                      | 72° F.  |  | 86° F.                  |  | 40° F.                     |  | 40° F.               |  | 40° F.                    |  |
|              |                                                      | 17,300  |  | 16,500                  |  | 15,200                     |  | 17,200               |  | 16,400                    |  |
| E            | Synthetic vitamin A acetate <sup>3</sup> , 200,000   | 17,300  |  | 16,500 (95)             |  | 16,300 (94)                |  | ...                  |  | 17,700 <sup>4</sup> (99)  |  |
| F            | U.S.P. Units per gram                                | 18,700  |  | 18,200 (97)             |  | 17,100 (91)                |  | ...                  |  | ...                       |  |
| F            | Fish oil concentrate                                 | 18,700  |  | 18,200 (97)             |  | 17,100 (91)                |  | 19,800 (106)         |  | 18,700 (100)              |  |
| F            | Synthetic vitamin A palmitate <sup>3</sup> , 400,000 | 17,000  |  | 17,000 (100)            |  | 17,300 (102)               |  | 19,700 (116)         |  | 18,900 (111)              |  |
| G            | U.S.P. units per gram                                | 14,700  |  | ...                     |  | 13,200 (90)                |  | ...                  |  | ...                       |  |
| G            | Fish oil concentrate                                 | 14,700  |  | ...                     |  | 13,200 (90)                |  | 14,600 (99)          |  | 16,300 (110)              |  |
| G            | Synthetic vitamin A palmitate <sup>3</sup> , 400,000 | 17,700  |  | ...                     |  | 14,800 (84)                |  | 17,700 (100)         |  | 17,000 <sup>4</sup> (107) |  |
| H            | U.S.P. Units per gram                                | 18,800  |  | ...                     |  | 16,300 (87)                |  | ...                  |  | ...                       |  |
| H            | Fish oil concentrate                                 | 18,800  |  | ...                     |  | 16,300 (87)                |  | 19,100 (102)         |  | 19,100 (102)              |  |
| H            | Synthetic vitamin A palmitate <sup>3</sup> , 200,000 | 17,900  |  | ...                     |  | 17,100 (96)                |  | 17,800 (99)          |  | 17,500 <sup>4</sup> (112) |  |
| H            | U.S.P. units per gram                                | 18,000  |  | ...                     |  | 18,100 (101)               |  | ...                  |  | ...                       |  |
| H            | Synthetic vitamin A acetate <sup>3</sup> , 200,000   | 18,000  |  | ...                     |  | 18,100 (101)               |  | 18,400 (102)         |  | 19,500 (108)              |  |
| H            | U.S.P. units per gram                                | 18,000  |  | ...                     |  | 18,100 (101)               |  | 18,400 (102)         |  | 19,500 (108)              |  |

<sup>1</sup>In most instances values represent single chemical assays.

<sup>4</sup>Biological values by U.S.P. rat assay method. Assays conducted by F. Jahns and associates, Roche Nutrition Laboratories.

<sup>5</sup>After 5 months of storage.

<sup>6</sup>After 7 months of storage.

periods.

3. A cottonseed oil dilution

Note: Figures in parentheses are percent retention based on initial assay value.



Retention of Added Vitamin A in Baked Foods  
Prepared with Synthetic A Fortified Margarine

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| Food Product                 | Added<br>Vitamin A | Vitamin A Content       |                                                                       | Retention<br>Percent |
|------------------------------|--------------------|-------------------------|-----------------------------------------------------------------------|----------------------|
|                              |                    | Uncooked<br>Preparation | After Cooking<br>(Moisture Corrected)<br>IU/100g. by Carr-Price Assay |                      |
| Chocolate cake               | 0.0                | ---                     | 0.0 <sup>a</sup>                                                      | ---                  |
| Chocolate cake               | 450                | 490                     | 450                                                                   | 93                   |
| Yellow cake                  | 540                | 620                     | 650                                                                   | 109                  |
| White cake                   | 0.0                | ---                     | 0.0 <sup>a</sup>                                                      | ---                  |
| White cake                   | 470                | 520                     | 570                                                                   | 107                  |
| Cookies, butterscotch slices | 0.0                | ---                     | 0.0 <sup>a</sup>                                                      | ---                  |
| Cookies, butterscotch slices | 1,120              | 1,000                   | 1,000                                                                 | 75                   |
| Cookies, vanilla drops       | 1,020              | 910                     | 1,180                                                                 | 93                   |
| White sauce, Test 1          | 400                | ---                     | 560                                                                   | 112 <sup>b</sup>     |
| White sauce, Test 2          | 390                | ---                     | 540                                                                   | 116 <sup>b</sup>     |
| White sauce, Test 3          | 360                | ---                     | 520                                                                   | 117 <sup>b</sup>     |
| Non-cooked frosting, Test 1  | 900                | 1,020                   | ---                                                                   | ---                  |
| Non-cooked frosting, Test 2  | 890                | 1,040                   | ---                                                                   | ---                  |
| Cooked frosting              | 390                | ---                     | 370                                                                   | 95                   |

<sup>a</sup>Yellow color (atypical) produced in Carr-Price assay which, if calculated as vitamin A, would amount to about 10 U.S.P. units per gram.

<sup>b</sup>Whole fluid milk used in the recipe probably enhanced vitamin A value.





Table 15

## Retention of Added Vitamin A to Peanut Butter

| <u>Storage<br/>Condition</u> | <u>Amount<br/>Added</u> | <u>Assay</u> | <u>%<br/>Added</u> |
|------------------------------|-------------------------|--------------|--------------------|
|                              | IU/lb                   |              |                    |
| Initial                      | 15,000                  | 16,000       | 107                |
| 3 mos/R.T.*                  | 15,000                  | 16,500       | 110                |
| 6 mos/R.T.                   | 15,000                  | 17,000       | 113                |
| 12 mos/R.T.                  | 15,000                  | 11,450       | 76                 |
| 3 wks/113°F                  | 15,000                  | 15,300       | 102                |
| 6 wks/113°F                  | 15,000                  | 12,500       | 83                 |
| 6 mos/86°F                   | 15,000                  | 14,400       | 96                 |
| 6 mos/98°F                   | 15,000                  | 12,000       | 80                 |

\* Room temperature 75°F (23°C)



### Retention of added vitamin A in stored nonfat dry milk

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Table 17

Biological Assay of Nonfat dry milk with added vitamins A and D<sub>2</sub>A. Biological availability of added vitamin A by rat assay<sup>a</sup>

| Material                        | No.<br>rats | Dose<br>per rat<br>(I. U. ) | Vitamin A                    |                               | Av.<br>deposited | Test<br><u>sample</u><br>Standard X100 |
|---------------------------------|-------------|-----------------------------|------------------------------|-------------------------------|------------------|----------------------------------------|
|                                 |             |                             | liver<br>storage<br>(I. U. ) | Vitamin A<br>deposited<br>(%) |                  |                                        |
| NRC Vit. A<br>standard          | 12          | 1100                        | 489                          | 44                            | 45               | 104                                    |
|                                 | 12          | 2200<br>(g)                 | 988                          | 45                            |                  |                                        |
| Fortified<br>nonfat dry<br>milk | 12          | 10 <sup>b</sup>             | 561                          | 51                            | 47               |                                        |
|                                 | 12          | 20 <sup>b</sup>             | 935                          | 43                            |                  |                                        |

B. Biological availability of added vitamin D<sub>2</sub> by rat assay<sup>c</sup>

| Material                            | No.<br>rats | Dose<br>per rat<br>(I. U. ) | Healing<br>response | Biovalue<br>(I. U. /g) | Claim<br>(I. U. /g) | Biovalue<br>Claim X100 |
|-------------------------------------|-------------|-----------------------------|---------------------|------------------------|---------------------|------------------------|
|                                     |             |                             |                     |                        |                     |                        |
| USP vit. D <sub>2</sub><br>standard | 10          | 1.40                        | 0.8                 | ...                    |                     |                        |
|                                     | 10          | 2.10                        | 1.3                 | ...                    |                     |                        |
|                                     | 10          | 3.16<br>(mg)                | 2.3                 | ...                    |                     |                        |
| Fortified<br>nonfat dry<br>milk     |             |                             |                     |                        |                     |                        |
| No. 1                               | 10          | 398                         | 1.6                 | 5.63                   | 5.29                | 106                    |
| No. 2                               | 10          | 398                         | 1.8                 | 6.33                   | 5.29                | 120                    |
| No. 3                               | 10          | 398                         | 1.5                 | 5.38                   | 5.29                | 102                    |

<sup>a</sup> Assay conducted as described in Anal. Chem. 20, 304 (1948); diet contained no added fat.

<sup>b</sup> Equivalent to 1100 and 2200 I. U by chemical assay

<sup>c</sup> Assay conducted as described in U. S. Pharmacopoeia 15, 889 (1955).



Table 18

Flavor evaluation of reconstituted milk  
from vitamin A fortified nonfat dry milk

| Sample                  | Any flavor score <sup>a</sup> |                  |
|-------------------------|-------------------------------|------------------|
|                         | Initial<br>Reconstitution     | 24<br>hours/40°F |
| After manufacture       |                               |                  |
| Wet method              | 1.6                           | 1.3              |
| Dry method              | 1.6                           | 1.3              |
| 8 months' storage/75°F  |                               |                  |
| Wet method              | 1.2                           | 1.2              |
| Dry method              | 1.5                           | 1.5              |
| 12 months' storage/75°F |                               |                  |
| Wet method              | 1.8                           | 1.8              |
| Dry method              | 1.5                           | 1.8              |

<sup>a</sup> Flavor evaluations made by a panel of 6 experienced tasters. Key: 5, pronounced vitamin off-flavor; 4, vitamin off-flavor; 3, slight vitamin off-flavor; 2, very slight vitamin off-flavor; 1, no vitamin off-flavor.





Table 19

Stability of vitamin A following reconstitution  
of vitamin A fortified nonfat dry milk

| Sample <sup>a</sup> | Reconstitution<br>storage<br>(hr at 40°F) | % vitamin<br>retention |     |
|---------------------|-------------------------------------------|------------------------|-----|
|                     |                                           | Wet                    | Dry |
| A                   | 24                                        | 102                    | 99  |
|                     | 72                                        | 101                    | 96  |
| B                   | 24                                        | 96                     | 95  |
|                     | 72                                        | 86                     | 89  |
| C                   | 24                                        | 99                     | 97  |
|                     | 72                                        | 93                     | 95  |
| D                   | 24                                        | 92                     | 98  |
|                     | 72                                        | 92                     | 96  |
| E                   | 24                                        | 96                     | 99  |
|                     | 72                                        | 92                     | 97  |

<sup>a</sup> Samples chosen for reconstitution studies already had a variable history of storage in the dry state, for example, sample A, 1 month at 75°F; sample C, 3 months at 98°F; and sample E, 12 months at 75°F. Values represent duplicate vitamin A assays.



Table 20

Packaging evaluation as judged by vitamin  
retention data

| Preference | Container                                                      | Vitamin A<br>retention |
|------------|----------------------------------------------------------------|------------------------|
|            |                                                                | (%)                    |
| 1          | Tin can, vacuum pack                                           | 96                     |
| 2          | Laminated pouches                                              | 92                     |
| 3          | Polyethylene bags, cardboard<br>cylinders                      | 90                     |
| 3          | Tin can, air pack                                              | 90                     |
| 4          | Cardboard cylinder, metal<br>ends                              | 89                     |
| 5          | Polyethylene bags, no<br>container (unprotected from<br>light) | 81                     |





Table 21

## Retention of vitamin A in various products

| Product                                       | Added<br>Vitamin A | After<br>Manufacture | After Storage                         |                         |                          |
|-----------------------------------------------|--------------------|----------------------|---------------------------------------|-------------------------|--------------------------|
|                                               |                    |                      | (IU vitamin A per ounce)              |                         |                          |
|                                               |                    |                      | 500 Hr.                               | 1,000 Hr.<br>at 113°F.  | 1,500 Hr.                |
| Confections <sup>2</sup>                      |                    |                      |                                       |                         |                          |
| Caramels <sup>2</sup>                         | 1,090              | 990                  | 1,060                                 | ...                     | 1,270                    |
| Milk Chocolate A <sup>3</sup>                 | 0                  | trace                | ....                                  | ....                    | ....                     |
| B <sup>3</sup>                                | 2,825              | 2,800                | 2,540                                 | 2,600                   | 2,640                    |
| C <sup>4</sup>                                | 3,325              | 3,400                | 3,100                                 | 3,000                   | 2,750                    |
|                                               | 3,325              | 3,200                | 3,050                                 | 3,050                   | 3,100                    |
| Chocolate Fudge <sup>1</sup>                  | 3,120              | 3,540                | 4,020                                 | 3,710                   | ...                      |
| Molasses Taffy <sup>1</sup>                   | 1,750              | 1,780                | 1,730                                 | ....                    | ....                     |
|                                               |                    |                      | 12 Months<br>at<br>72°F.              | 6 Months<br>at<br>86°F. | 12 Months<br>at<br>98°F. |
| Caramels                                      | 1,090              | 990                  | 1,190                                 | ...                     | 1,160                    |
| Milk Chocolate A <sup>3</sup>                 | 2,825              | 2,800                | 1,930                                 | 2,180                   | 1,920                    |
| B <sup>3</sup>                                | 3,325              | 3,400                | 2,440                                 | 2,520                   | 2,260                    |
| C <sup>4</sup>                                | 3,325              | 3,200                | 2,660                                 | 2,550                   | 2,240                    |
|                                               |                    |                      |                                       | 6 Months<br>at<br>72°F. | 12 Months<br>at<br>72°F. |
| Pediatric and Geriatric Products <sup>3</sup> |                    |                      |                                       |                         |                          |
| Dry milk or modified<br>milk products A       | ...                | 2,130                | 2,000                                 | 1,940                   | 1,970                    |
| B                                             | ...                | 1,620                | 1,730                                 | ...                     | 1,840 <sup>5</sup>       |
| C                                             | ...                | 1,210                | 1,030                                 | 990                     | 1,030                    |
|                                               |                    |                      | (IU vitamin A per pint <sup>6</sup> ) |                         |                          |
|                                               |                    |                      |                                       | 6 Months<br>at<br>72°F. | 12 Months<br>at<br>72°F. |
| Canned liquid milk<br>products A              | ...                | 3,200                | 3,210                                 | 3,325                   | ...                      |
| B                                             | ...                | 1,620                | 1,500                                 | 1,510                   | 1,390                    |
| C                                             | ...                | 4,450                | 4,150                                 | ...                     | 3,500 <sup>5</sup>       |
| D                                             | ...                | 1,430                | 1,390                                 | 1,250                   | 1,290                    |
|                                               |                    |                      | 6 Weeks<br>at 0°F.                    | 24 Weeks<br>at 0°F.     |                          |
| Ice Cream <sup>7</sup>                        |                    |                      |                                       |                         |                          |
| Trial A                                       | 0                  | 1,300                | ...                                   | ...                     |                          |
| B <sup>4</sup>                                | 3,320              | 4,260                | 4,040                                 | 4,800                   |                          |
| C <sup>3</sup>                                | 3,320              | 4,120                | 4,500                                 | 4,600                   |                          |
| D <sup>3</sup>                                | 4,800              | 6,200                | 5,240                                 | 5,740                   |                          |

Footnotes on next page



Table 21

## Footnotes

<sup>1</sup>As determined by Carr-Price assay

<sup>2</sup>Vitamin A palmitate, type Pl. 3, 1,300,000 U.S.P. units per gram

<sup>3</sup>Vitamin A palmitate, type PIMO, 1,000,000 U.S.P. units per gram in oil.

<sup>4</sup>Vitamin A acetate, type AIMO, 1,000,000 U.S.P. units per gram in oil.

<sup>5</sup>14 months storage.

<sup>6</sup>Vitamin A per pint as consumed

<sup>7</sup>12 percent butterfat, 11 percent serum solids, 17 percent sugar,  
0.3 percent stabilizer (1 pint equals 282g).





Table 22

Vitamin A content (IU/3 oz) of fortified potato flakes, packed in air and under nitrogen, after storage at different temperatures.<sup>a</sup> (65)

| Storage<br>(weeks) | Air pack |       |       | N <sub>2</sub> pack |       |       |
|--------------------|----------|-------|-------|---------------------|-------|-------|
|                    | 75°F     | 98°F  | 113°F | 75°F                | 98°F  | 113°F |
| 0                  | 10,900   | ———   | ———   | 10,900              | ———   | ———   |
| 4                  | 9,900    | ———   | ———   | 10,900              | ———   | ———   |
| 7                  | ———      | ———   | 7,600 | ———                 | ———   | 7,900 |
| 10                 | ———      | ———   | 7,100 | ———                 | ———   | 7,500 |
| 12                 | ———      | 8,350 | ———   | ———                 | 9,250 | ———   |
| 16                 | 8,000    | 7,000 | 6,050 | 10,600              | 8,150 | 7,400 |
| 28                 | 7,420    | ———   | ———   | 10,600              | ———   | ———   |

<sup>a</sup> Initial value of 10,900 IU/3 oz represents a recovery of 10,900 IU/3 oz; or 91% of 12,000 IU/3 oz added before drying.



Table 23

## Retention of Added Vitamin A in Tea Dust and Tea Leaves (67)

| Tea type | Form of vitamin A                                                          | How applied to tea | Storage conditions | Percent retention |
|----------|----------------------------------------------------------------------------|--------------------|--------------------|-------------------|
| Dust     | Palmitate 250 SD powder                                                    | Dry mixing         | 1 yr, room temp.   | 85                |
| Leaves   | Palmitate or acetate emulsion diluted in water <sup>a</sup>                | Sprayed            | 1 mo, room temp.   | 50                |
| Leaves   | Palmitate or acetate emulsion diluted in 20% dextrin solution <sup>a</sup> | Sprayed            | 1 mo, room temp.   | 85                |
| Leaves   | Palmitate or acetate emulsion diluted in 20% dextrin solution <sup>a</sup> | Sprayed            | 6 mo, room temp.   | 43                |
| Leaves   | Palmitate or acetate emulsion diluted in 20% dextrin solution <sup>a</sup> | Sprayed            | 6 mo, 37°C         | 20                |
| Leaves   | Palmitate or acetate emulsion diluted in 50% sucrose solution <sup>a</sup> | Sprayed            | 1 mo, 37°C         | 98                |
| Leaves   | Palmitate or acetate emulsion diluted in 50% sucrose solution <sup>a</sup> | Sprayed            | 6 mo, 37°C         | 90                |

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<sup>a</sup> 2 g of emulsion diluted with 98 g of solution; 20-100  $\mu$ l of resulting solution (equivalent to 200-1,000 IU of vitamin A) sprayed onto 5 g of leaves, which were then dried at 60°C for 1 hr





Table 24

## Retention of Vitamin A in Brewed Tea (67)

| Tea type | Form of Vitamin A                                | How applied to tea | Percent recovery in brewed tea |                |
|----------|--------------------------------------------------|--------------------|--------------------------------|----------------|
|          |                                                  |                    | 5-min cooking                  | 1-hour cooking |
| Dust     | Palmitate 250 SD powder                          | Dry mixing         | 100                            | 100            |
| Leaves   | Palmitate special water-soluble oil <sup>a</sup> | Sprayed            | 45                             | 30             |
| Leaves   | Palmitate emulsion <sup>a</sup>                  | Sprayed            | 100                            | 100            |
| Leaves   | Acetate emulsion <sup>a</sup>                    | Sprayed            | 50                             | 4              |

<sup>a</sup> Diluted in 50% sucrose solution



Table 25

Results of Taste Testing Trials of Sugar Fortified with Vitamin A with the Triangle Method<sup>1</sup> (68)

| Food                                    | Answers |           | Flavor was:    | With      |    | Without |
|-----------------------------------------|---------|-----------|----------------|-----------|----|---------|
|                                         | Correct | Incorrect |                | Vitamin A |    |         |
| Coffee (hot)                            | 17      | 13        | Detectable     | 12        | 18 |         |
| INCAPARINA' (hot)                       | 11      | 19        | Not detectable | 18        | 12 |         |
| Pineapple refreshment<br>(fresh) (cold) | 22      | 8         | Detectable     | 24        | 6  |         |
| Tea (hot)                               | 10      | 20        | Not detectable | 18        | 12 |         |
| Orangeade (cold)                        | 18      | 12        | Detectable     | 18        | 12 |         |
| Lemonade (cold)                         | 14      | 16        | Not detectable | 14        | 16 |         |
| Horchata (rice flour cold drink)        | 11      | 19        | Not detectable | 13        | 17 |         |

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<sup>1</sup>Fundamentals of Quality Control for the Food Industry, by Kramer, A., and Twigg, B.A. The Avi Publishing Co., Inc. Westport, Connecticut, 1966.





Table 26

Retinol Content of Breast Milk from Women Consuming  
Sugar With and Without Retinol Palmitate (68)

| Mos. of<br>Lactation | Retinol (mcg/100 ml)                  |               |
|----------------------|---------------------------------------|---------------|
|                      | No.<br>cases                          | No.<br>cases  |
| 0-3                  | 5 <sup>1</sup> 66.6±17.6 <sup>2</sup> | 3 22.2±1.6*   |
| 4-6                  | 5 30.4± 7.8                           | 6 22.7±5.0    |
| 7-9                  | 4 39.4±21.1                           | 4 12.4±2.4**  |
| > 9                  | 10 30.1±0.4                           | 3 19.4±6.4**  |
| All groups           | 24 39.3±5.7                           | 16 19.4±2.4** |

<sup>1</sup>Number of cases

<sup>2</sup>Mean ± Standard error

\*P < 0.05

\*\*P < 0.01



Table 27

## Cost Analysis Form (72)

## OBVIOUS COSTS OF IN-HOUSE PREMIX

|                             |    |
|-----------------------------|----|
| Micronutrients.....         | \$ |
| Labor for premixing.....    | \$ |
| Equipment and overhead..... | \$ |
| Analytical charges.....     | \$ |
| Packaging costs.....        | \$ |

## OTHER RELATED COSTS OF IN-HOUSE PREMIX

|                                               |    |
|-----------------------------------------------|----|
| Purchasing of single ingredients.....         | \$ |
| Developmental costs of premix.....            | \$ |
| Analytical charges on single ingredients..... | \$ |
| Product loss of single ingredients.....       | \$ |
| Warehousing costs of single ingredients.....  | \$ |
| Other supporting costs.....                   | \$ |

Total cost of in-house premix \$

## COST COMPARISON

|                                 | COST PER LB.<br>PREMIX | COST PER LB.<br>FOOD PRODUCT |
|---------------------------------|------------------------|------------------------------|
| In-House Premix.....            | \$                     | \$                           |
| Commerically Prepared<br>Premix | \$                     | \$                           |
| Difference                      | \$                     | \$                           |





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